



Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

Prof. Dr. Vladimir Zaichick, PhD, DSc, CChem, FRSC

Department of Radionuclide Diagnostics,
Medical Radiological Research
Center, Obninsk, Russia



Abstract

Background: 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.

Objectives: 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 ChEs such as silver (Ag), calcium (Ca), chlorine (Cl), cobalt (Co), chromium (Cr), copper (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 “normal” thyroid (NT) of 105 individuals as well as in nodular tissue of thyroids with TBN (79 patients) and to TMN (41 patients). Measurements were performed using a combination of non-destructive methods X-ray fluorescence and instrumental neutron activation analysis with high resolution spectrometry of short- and long-lived radionuclides.

Results: It was observed that in TMN tissue the mean mass fraction of I was lower while the mean mass fractions of K, Mg, and Rb were higher than in NT and TBN tissue. It was demonstrated that I content in nodular tissue is the most informative parameter for the diagnosis of thyroid malignancy. It was found that “Sensitivity”, “Specificity” and “Accuracy” of TMN identification using the I level in the needle biopsy of affected thyroid tissue was significantly higher than that using US examination and cytological test of fine needle aspiration biopsy.

Conclusions: It was concluded that determination of the I level in a needle biopsy of TNs using non-destructive instrumental analytical method is a fast, reliable, and very informative diagnostic tool that can be successfully used as an additional test of thyroid malignancy identification.

Keywords: Diagnosis of thyroid malignancy; Normal thyroid; Thyroid nodules; Chemical elements; X-ray fluorescence; Neutron activation analysis

Copyright : © 2021 The Authors. Published by Medical Editor and Educational Research Publishers Ltd. This is an open access article under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. 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

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

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 ChEs have also essential physiological role and involved in thyroid functions [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of ChEs depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the ChEs may

disturb the cell functions and may result in cellular proliferation, degeneration, death, benign or malignant transformation [13-15].

In our previous studies the complex of *in vivo* and *in vitro* nuclear analytical and related methods was developed and used for the investigation of iodine and other ChEs contents in the normal and pathological thyroid [16-22]. Iodine level in the normal thyroid was investigated in relation to age, gender and some non-thyroidal diseases [23,24]. After that, variations of many ChEs content with age in the thyroid of males and females were studied and age- and gender-dependence of some ChEs was observed [25-41]. Furthermore, a significant difference between some ChEs contents in colloid goiter, thyroiditis, thyroid adenoma, and cancer in comparison with normal thyroid and thyroid tissue adjacent to TNs was demonstrated [42-48].

The present study had two aims. The main objective was to assess the 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 “normal” thyroid (NT) as well as 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 evaluate ChEs content to aid diagnosis of thyroid malignancy.

2. MATERIAL AND METHODS

Samples of the NT were obtained from randomly selected autopsy specimens of 105 deceased (European-Caucasian, mean age 44 ± 21 years, range 2-87), who had died suddenly. The majority of deaths were due to trauma. All the deceased were citizens of Obninsk and had undergone

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

routine autopsy at the Forensic Medicine Department of City Hospital, Obninsk. A histological examination in the control group was used to control the age norm conformity, as well as to confirm the absence of micro-nodules and latent cancer.

All patients suffered from TBN (n=79, mean age $M \pm SD$ was 44 ± 11 years, range 22-64) and from TMN (n=41, mean age $M \pm SD$ was 46 ± 15 years, range 16-75) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre (MRRC), Obninsk. Thick-needle puncture biopsy of suspicious nodules of the thyroid was performed for every patient, to permit morphological study of thyroid tissue at these sites and to estimate their 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 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 NT, TBN and TMN were divided into two portions using a titanium scalpel to prevent contamination by ChEs of stainless steel [49]. One was used for morphological study while the other was intended for ChEs analysis. After the samples intended for TEs analysis were weighed, they were freeze-dried and homogenized [50].

To determine the contents of the ChEs by comparison with known data for standard, aliquots of commercial, chemically pure compounds and synthetic reference materials were used [51]. 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) 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 ^{109}Cd radionuclide, methods of analysis and the results of quality control were presented in our earlier publications concerning the EDXRF of ChEs contents in human thyroid [25,26].

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 ChEs contents in human thyroid [27,28] and prostate [53].

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 ChEs contents in human thyroid [29,30] and prostate [52].

All samples for ChEs analysis were prepared in duplicate, and mean values of ChEs contents were used in final calculation. Mean values of ChEs

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

contents were used in final calculation for the Br, Fe, Rb, and Zn mass fractions measured by two methods. Using Microsoft Office Excel software, some basic statistics, including, arithmetic mean, standard deviation of mean, standard error of mean, minimum and maximum values (range) was calculated for ChEs contents in three groups of thyroid tissue (NT, TBN and TMN). The difference in the results between three groups of samples was evaluated by the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

3. RESULTS

Table 1 depicts certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, range) 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 tissue samples of three groups – NT, 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 NT and TBN, NT and TMN, and also TBN and TMN is presented in Table 2.

Fig. 1 depicts individual data sets for I, K, Mg, and Rb mass fraction in all samples of NT, TBN, and TMN group.

Parameters of the sensitivity, specificity and accuracy ($M \pm 95\%$ confidence interval) of using I mass fraction for the diagnosis of thyroid malignancy are presented in Table 3. An estimation was made from comparison individual values in TMN group with those in NT and TBN groups combined, if value of I mass fraction equals 145 mg/kg dry tissue was chosen as upper limit (cut off) for thyroid malignancy.

Table 1. Basic 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 normal thyroid (N) and in thyroid benign (TBN) and malignant (TMN) nodules

El	NT, n=105		TBN, n=79		TMN, n=41	
	Mean \pm SD(SEM)	Range	Mean \pm SD(SEM)	Range	Mean \pm SD(SEM)	Range
Ag	0.015 \pm 0.014 (0.002)	0.0012-0.080	0.226 \pm 0.219(0.031)	0.0020-0.87	0.193 \pm 0.215(0.041)	0.0075-1.02
Br	14.9 \pm 11.0(1.2)	1.90-54.1	412 \pm 682(98)	3.20-2628	139 \pm 203(36)	6.20-802
Ca	1711 \pm 1022(109)	414-6230	1237 \pm 902(138)	52.0-4333	2397 \pm 2368(558)	452-8309
Cl	3400 \pm 1452(174)	1030-6000	8231 \pm 3702(772)	1757-16786	7699 \pm 2900(703)	4214-14761
Co	0.040 \pm 0.027(0.003)	0.0046-0.140	0.062 \pm 0.033(0.005)	0.0083-0.16	0.055 \pm 0.031(0.006)	0.0042-0.14
Cr	0.539 \pm 0.272(0.032)	0.130-1.30	0.966 \pm 0.844(0.121)	0.075-3.65	0.835 \pm 0.859(0.157)	0.0390-3.50
Cu	4.23 \pm 1.52(0.18)	0.500-7.50	10.2 \pm 9.2(1.7)	2.90-35.2	14.5 \pm 9.4(2.6)	4.00-32.6
Fe	223 \pm 93(10)	51.0-512	387 \pm 475(56)	52,3-2563	243 \pm 177(29)	55.1-887
Hg	0.042 \pm 0.036(0.004)	0.0065-0.180	0.924 \pm 0.649(0.088)	0.0817-3.01	0.824 \pm 0.844(0.149)	0.0685-3.75
I	1841 \pm 1027(107)	114-5061	991 \pm 906(105)	29.0-3906	71.8 \pm 62.0(10)	2.00-261
K	6071 \pm 2773(306)	1740-14300	6191 \pm 2360(352)	797-12222	9655 \pm 4444(970)	1660-19225
Mg	285 \pm 139(17)	66.0-930	331 \pm 180(26)	13.0-844	450 \pm 232(51)	122-1033
Mn	1.35 \pm 0.54(0.07)	0.510-4.18	1.80 \pm 1.38(0.21)	0.100-5.54	1.90 \pm 1.41(0.32)	0.100-5.79
Na	6702 \pm 1764(178)	3050-13453	10207 \pm 3786(558)	2319-22381	85562959(646)	4083-17284
Rb	8.16 \pm 4.55(0.49)	1.66-29.4	9.16 \pm 4.21(0.50)	1.00-20.3	12.6 \pm 4.6(0.7)	5.50-27.4
Sb	0.111 \pm 0.072(0.008)	0.0047-0.308	0.137 \pm 0.116(0.016)	0.0024-0.47	0.124 \pm 0.081(0.015)	0.0160-0.381
Sc	0.005 \pm 0.004(0.001)	0.0002-0.014	0.014 \pm 0.022(0.003)	0.0002-0.09	0.008 \pm 0.013(0.002)	0.0002-0.0565
Se	2.32 \pm 1.29(0.14)	0.439-5.80	2.75 \pm 2.13(0.29)	0.720-12.6	2.04 \pm 1.02(0.18)	0.143-4.70

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

Sr	4.55±3.22(0.37)	0.100-13.7	4.48±6.84(0.88)	0.42-32.0	6.25±7.83(1.63)	0.930-30.8
Zn	105.1±40.1(4.3)	7.10-221	115.3±49.6(5.9)	47.0-270	89.7±57.6(10.8)	36.7-326

El – element, M – arithmetic mean, SD – standard deviation, SEM – standard error of mean, Range – min and max values

Table 2. Ratio of means and the difference between mean values 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 tissue) in normal thyroid (NT) and in thyroid benign (TBN) and malignant (TMN) nodules

El	TBN and NT			TMN and NT			TMN and TBN		
	Ratio TBN/NT	<i>p</i> t-test	<i>p</i> U-test	Ratio TMN/NT	<i>p</i> t-test	<i>p</i> U-test	Ratio TMN/TBN	<i>p</i> t-test	<i>p</i> U-test
Ag	15.0	<0.00001	≤0.01	12.8	0.00022	≤0.01	0.85	0.515	>0.05
Br	27.7	0.00020	≤0.01	9.33	0.0017	≤0.01	0.34	0.012	≤0.01
Ca	0.72	0.0082	≤0.01	1.40	0.243	>0.05	1.94	0.058	≤0.05
Cl	2.42	<0.00001	≤0.01	2.26	<0.00001	≤0.01	0.94	0.614	>0.05
Co	1.54	0.00016	≤0.01	1.38	0.022	≤0.01	0.89	0.370	>0.05
Cr	1.79	0.0012	≤0.01	1.55	0.073	≤0.05	0.86	0.511	>0.05
Cu	2.41	0.0018	≤0.01	3.43	0.0019	≤0.01	1.42	0.176	>0.05
Fe	1.74	0.0055	≤0.01	1.09	0.519	>0.05	0.63	0.026	≤0.01
Hg	21.9	<0.00001	≤0.01	19.6	<0.00001	≤0.01	0.89	0.567	>0.05
I	0.54	<0.00001	≤0.01	0.039	<0.00001	≤0.01	0.072	<0.00001	≤0.01
K	1.02	0.797	>0.05	1.59	0.0017	≤0.01	1.56	0.0025	≤0.01
Mg	1.16	0.140	>0.05	1.58	0.0047	≤0.01	1.36	0.045	≤0.01
Mn	1.33	0.048	≤0.01	1.41	0.107	>0.05	1.06	0.794	>0.05
Na	1.52	<0.00001	≤0.01	1.28	0.011	≤0.01	0.84	0.059	≤0.05
Rb	1.12	0.153	>0.05	1.54	<0.00001	≤0.01	1.38	0.00022	≤0.01
Sb	1.23	0.143	>0.05	1.12	0.423	>0.05	0.91	0.572	>0.05
Sc	3.13	0.0054	≤0.01	1.67	0.223	>0.05	0.53	0.105	>0.05
Se	1.19	0.174	>0.05	0.88	0.235	>0.05	0.74	0.039	≤0.01
Sr	0.98	0.948	>0.05	1.37	0.319	>0.05	1.40	0.348	>0.05
Zn	1.10	0.163	>0.05	0.85	0.191	>0.05	0.78	0.042	≤0.01

El – element, *t*-test - Student’s *t*-test, U-test - Wilcoxon-Mann-Whitney *U*-test, **Bold** significant differences

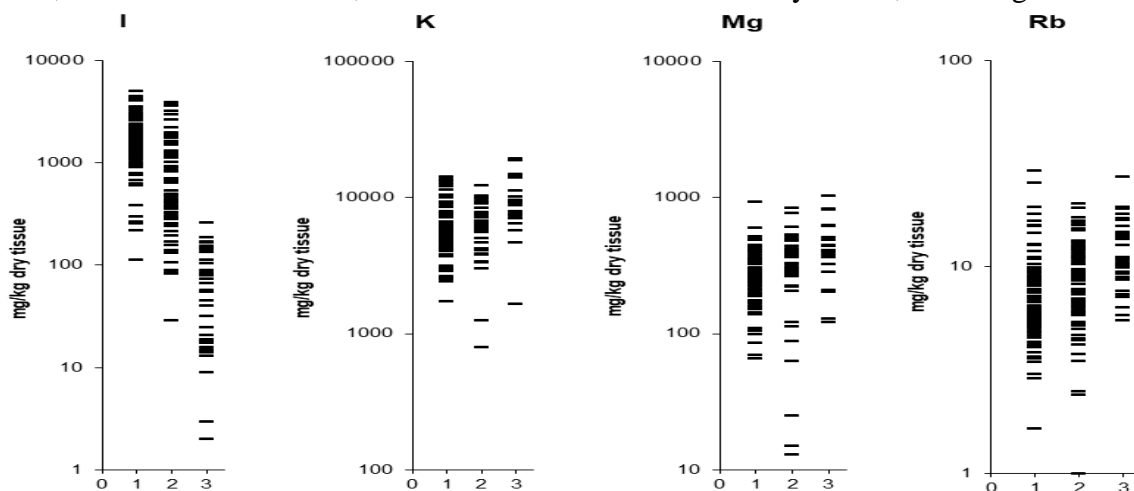


Fig.1. Individual data sets for I, K, Mg, and Rb mass fractions in samples of normal thyroid (1), thyroid benign nodules (2) and thyroid malignant nodules (3).

Table 3. Parameters of the sensitivity, specificity and accuracy (M±95% confidence interval) of I mass fraction for the diagnosis of TMN (an estimation is made for “TMN or NT and TBN”)

Element	Upper limit for TMN (cut off)	Sensitivity %	Specificity %	Accuracy %
I	145 mg/kg dry tissue	84±6	96±2	94±2

NT - normal thyroid, TBN - thyroid benign nodules, TMN- thyroid malignant nodules

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

The comparison of our results with published data (from 1990 year) for I mass fraction in NT [27,28,31-34,37,53-72], TBN [54,56,57,62,63,67-80], and TMN [54,56,57,,60, 64-66,73,74,81-85] is shown in Tables 4, 5, and 6, respectively. 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%) [86] and ash (4.16% on dry mass basis) [87] contents in thyroid of adults.

4. DISCUSSION

As was shown before [25-30,52] 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 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 NT, TBN, and TMN groups of tissue samples presented in Tables 1-3 and Figure 1.

From Table 2, it is observed that in TMN tissue the mass fractions of I is significantly lower while the mass fraction of K, Mg, and Rb is higher than

in both NT and TBN groups. However, as illustrated in Figure 1, I content is the most informative parameter for the diagnosis of TMN (Fig. 1). If the I level of 145 mg/kg dry tissue (about M+SD for TMN group) is chosen as the upper limit (cut off) for TMN tissue (Fig.1), results for a “malignant or non- malignant” determination from results obtained would be the following:

$$\text{Sensitivity} = \{ \text{correct positive test (CPT)} / [\text{CPT} + \text{false negative test (FNT)}] \} \times 100\% = 84 \pm 6\%;$$

$$\text{Specificity} = \{ \text{correct negative test (CNT)} / [\text{CNT} + \text{false positive test (FPT)}] \} \times 100\% = 96 \pm 2\%;$$

$$\text{Accuracy} = [(\text{CPT} + \text{CNT}) / (\text{CPT} + \text{FNT} + \text{CNT} + \text{FPT})] \times 100\% = 94 \pm 2\%.$$

The number of people examined was taken into account for calculation of confidence intervals [88]. In other words, if I contents in a nodule biopsy sample do not exceed 145 mg/kg dry tissue, one could diagnose a malignant tumor with an accuracy of 94±2%. Using the I-test makes it possible to diagnose thyroid malignancy in 84±6% cases (sensitivity).

Table 4. Reference data of I mass fractions in “normal” human thyroid published from 1990 year

Reference	Method	n	Age, years M(Range)	Sample preparation	I, mg/kg dry tissue	
					M±SD	Range
Handl et al. 1990 [53]	Chem	39	21-86	-	1276±664	-
Aeschimann et al.1994 [54]	Chem	1	-	AD	2028	-
Boulyga et al. 1997 [55]	NAA	29	-	D, A	1778±381	-
	NAA	10	-	D, A	1905±635	-
Boulyga et al. 1999 [56]	NAA	12	-	D, A	-	800-2950
Reddy et al. 2002 [57]	PIXE	4	-	D, Press	916±88	-
Wang et al. 2002 [58]	-	21	Adult	-	2712±800	-
Murillo et al. 2005 [59]	Color	5	30-43	AD	948-3356	948-3356
Hansson et al. 2008 [60]	EDXRF	10	57-80	Intact	2400	1200-4800
Zabala et al. 2009 [61]	SFI	50	17-60	AD	5772±2708	1676-13720
Zhu et al. 2010 [62]	ICPMS	50	20-60	AD	2648	964-4760
Błazewicz et al. 2011 [63]	IC	50	M=25	Fixed	601±192	624-4020
				Frozen	623±187	840 -4000
Zaichick et al. 2017a[27]	NAA	72	2-80	Intact	1786±940	220-4205
Zaichick et al. 2017b[28]	NAA	33	3.5-87	Intact	1956±1199	114-5061
Zaichick et al. 2018a [31]	EDXRF,NAA	72	2-80	Intact	1786±940	220-4205
Zaichick et al. 2018b[32]	EDXRF,NAA	33	3.5-87	Intact	1956±1199	114-5061
Zaichick et al. 2018c[33]	NAA,ICPAES	33	3.5-87	Intact	1956±1199	114-5061
Zaichick et al. 2018d [34]	NAA,ICPAES	72	2-80	Intact	1786±940	220-4205

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

Zaichick et al. 2018e[37]	NAA	105	2-80	Intact	1841±1027	114-5061
Zaichick et al. 2018f[64]	NAA	105	44±21	Intact	1841±1027	114-5061
Zaichick et al. 2018g[65]	NAA	105	2-80	Intact	1841±1027	114-5061
Zaichick et al. 2018h[66]	NAA	105	44±21	Intact	1841±1027	114-5061
Zaichick 2021a[67]	NAA	105	2-87	Intact	1841±1027	114-5061
Zaichick 2021b[68]	NAA	105	44±21	Intact	1841±1027	114-5061
Zaichick 2021c[69]	NAA	105	2-87	Intact	1841±1027	114-5061
Zaichick 2021b[70]	NAA	105	44±21	Intact	1841±1027	114-5061
Zaichick 2021a[71]	NAA,ICPAES	105	2-87	Intact	1841±1027	114-5061
Zaichick 2021b[72]	NAA,ICPAES	105	44±21	Intact	1841±1027	114-5061
Median of means		1841				
Range of means (M_{\min} - M_{\max}),		601 – 5772				
Ratio M_{\max}/M_{\min}		9.6				
All references		27				

M – arithmetic mean, SD – standard deviation of mean,

Chem – chemical method, NAA – neutron activation analysis, PIXE – proton induced X-ray fluorescent emission, Color – colorimetric method, EDXRF – energy dispersive X-ray fluorescent analysis, SFI - spectrophotometric flow injection method , ICPMS – inductively coupled plasma mass spectrometry, IC - ion chromatography , ICPAES – inductively coupled plasma atomic emission spectrometry,

AD – acid digestion, D – drying at high temperature, A – ashing, AD – acid digestion.

Table 5. Reference data of I mass fractions in thyroid benign nodules published from 1990 year

Reference	Method	n	Age, years M(Range)	Sample preparation	I, mg/kg dry tissue	
					M±SD	Range
Nishita et al. 1990 [73]	NAA	14	28-71	Washed	396±74	66-1028
	NAA	7	18-74	Washed	115±40	21-344
Aeschmann et al.1994 [54]	Chem	11	-	AD	516	92-3548
Bellisola et al. 1998 [74]	NAA	20	17-82	Washed	660 ±360	560 -910
	NAA	22		Washed	1140 ±1640	7 - 3810
	NAA	12		Washed	640 ±660	3 - 1840
	NAA	6		Washed	130 ± 120	4 - 330
Boulyga et al. 1999 [56]	NAA	19	-	Washed -	-	100-4050
Reddy et al. 2002 [57]	PIXE	4	-	D, Press	888±88	-
Zhu et al.. 2010 [62]	ICPMS	50	20-60	AD	2648	964-4760
Błazewicz et al. 2011 [63]	IC	50	M=25	Fixed	601±192	624-4020
	IC	50		Frozen	623±187	840 -4000
	IC	66	M=35	Fixed	77±14	41-104
Zaichick 2021 [67]	NAA	46	30-64	Intact	1141±931	29-3715
Zaichick 2021 [68]	NAA	19	41±11	Intact	961±1013	131-3906
Zaichick 2021 [69]	NAA	8	40±10	Intact	951±630	83-1787
Zaichick 2021 [70]	NAA	6	39±9	Intact	276±283	85-824
Zaichick 2021 [71]	NAA,ICPAES	46	30-64	Intact	1141±931	29-3715
Zaichick 2021 [72]	NAA,ICPAES	19	41±11	Intact	961±1013	131-3906
Zaichick 2021 [75]	EDXRF,NAA	46	30-64	Intact	1144±943	29-3715
Zaichick 2021 [76]	EDXRF,NAA	19	22-55	Intact	962±1013	131-3906
Zaichick 2021 [77]	EDXRF,NAA	8	34-55	Intact	951±630	83-1787
Zaichick 2021 [78]	NAA	6	34-50	Intact	276±283	85-824
Zaichick 2022 [79]	EDXRF	79	22-64	Intact	1107±1358	47-8260
Zaichick 2022 [80]	NAA,ICPAES	79	22-64	Intact	1086±1219	29-8260
Median of means		920				
Range of means (M_{\min} - M_{\max}),		77 – 2648				
Ratio M_{\max}/M_{\min}		34.4				
All references		20				

M – arithmetic mean, SD – standard deviation of mean,

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

NAA – neutron activation analysis, Chem – chemical method, PIXE – proton induced X-ray fluorescent emission, ICPMS – inductively coupled plasma mass spectrometry, IC – ion chromatography, ICPAES – inductively coupled plasma atomic emission spectrometry, EDXRF – energy dispersive X-ray fluorescent analysis,

AD – acid digestion

Thus, I content in a nodule biopsy as biomarker of TMN could become a powerful diagnostic tool.

To a large extent, the resumption of the search for new methods for diagnosis of TMN was due to experience gained in a critical assessment of the limited capacity of US examination and cytological test of fine needle aspiration biopsy [2-4]. In addition to the US examination and morphological study of needle-biopsy of the thyroid nodules, the I-test developed in the present study seems to be very useful. Experimental conditions of the present study were approximated to the hospital conditions as closely as possible. In all cases a part of the material obtained from a puncture needle biopsy of the affected site in the thyroid was analyzed. Therefore, our data allow us to evaluate adequately the importance of the I-test for the diagnosis of TMN. Obtained characteristics for accuracy, sensitivity, and specificity of the I-test 94, 96, and 84,

respectively, are significantly better than these parameters of the US examination (nearly 80%) [2,3]. At that, the I-test gives a definite conclusion for all nodules investigated while using the morphological study of needle-biopsy up to 30% of nodules are categorized as cytologically “indeterminate” [4].

Mean values obtained for I contents in NT, TBN, and TMN agree well with median of mean values published in scientific literature for period from 1990 up to 2022 year (Table 4, 5, and 6, respectively). The range of means of I level reported in the literature for NT, TBN, and TMN vary widely (Tables 4-6). This can be explained by a dependence of I 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 I are lost as a result of this treatment [89-91].

Table 6. Reference data of I mass fractions in thyroid malignant nodules published from 1990 year

Reference	Method	n	Age, years M(Range)	Sample preparation	I, mg/kg dry tissue	
					M±SD	Range
Nishida et al 1990 [73]	NAA	8	21-67	Washed	≤23±10	<DL-67
Aeschmann et al 1994 [54]	Chem	4	-	AD	40	16-140
Bellisola et al 1998 [74]	NAA	12	17-82	Washed	200±210	6 - .430
Boulyga et al 1999 [56]	NAA	19	-	-	-	32-900
Reddy et al 2002 [57]	PIXE	4	-	D, Press	<30	-
Hansson et al 2008 [60]	EDXRF	7	21-58	Intact	<400	-
Zaichick et al. 2018a [64]	NAA	41	16-75	Intact	71.8±62	2-261
Zaichick et al. 2018b [65]	EDXRF,NAA	41	46±15	Intact	71.8±62	2-261
Zaichick et al. 2018c [66]	NAA,ICPAES	41	16-75	Intact	71.8±62	2-261
Zaichick. 2022a [81]	EDXRF	41	16-75	Intact	71.6±72.5	2-341
Zaichick. 2022b [82]	NAA	41	16-75	Intact	71.8±62	2-261
Zaichick. 2022c [83]	NAA	41	16-75	Intact	71.8±62	2-261
Zaichick. 2022d [84]	EDXRF,NAA	41	16-75	Intact	71.8±62	2-261
Zaichick. 2022e [85]	NAA,ICPAES	41	16-75	Intact	71.8±62	2-261

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

Median of means	71.8
Range of means ($M_{\min} - M_{\max}$),	23 – 400
Ratio M_{\max}/M_{\min}	17.4
All references	14

M – arithmetic mean, SD – standard deviation of mean,

NAA – neutron activation analysis, Chem – chemical method, PIXE – proton induced X-ray fluorescent emission, EDXRF – energy dispersive X-ray fluorescent analysis, ICPAES – inductively coupled plasma atomic emission spectrometry,

AD – acid digestion, D – drying at high temperature

It is well known that compared to other soft tissues, the human thyroid gland has significantly higher levels of I, because this element plays an important role in its normal functions, through the production of thyroid hormones (thyroxin and triiodothyronine) which are essential for cellular oxidation, growth, reproduction, and the activity of the central and autonomic nervous system. As was shown in present study, malignant transformation is accompanied by a significant loss of tissue-specific functional features, which leads to a drastically reduction in I content associated with functional characteristics of the human thyroid tissue. However, it is necessary to keep in mind that biochemical, or in other words, functional changes in thyroid cells are present from the earliest development of malignancy, which precedes any histopathological indication of malignancy, and these biochemical changes persist during progression of the malignancy and remain present in advanced thyroid cancer. Thus, I depletion is an early step in the malignant proliferation process and I depletion in nodular tissue precedes the morphological transformation of cells from being histopathologically benign to malignant.

In our study non-destructive INAA-SLR was used for I determination. This method needs in using a nuclear reactor that is not always available in clinical practice. However there is an alternative non-destructive method such as EDXRF analysis, including “the total reflection” version (TRXRF),

which allows reliable determinations of I and many other ChEs contents in a microprobe of a human body tissues and fluids within a few minutes [92]. EDXRF is a fully instrumental and non-destructive method because sample is investigated without requiring any pretreatment or its consumption. Moreover, it is well known that among the most modern analytical technologies, EDXRF is one of the simplest, fastest, most reliable and efficient of the available techniques for ChEs determination [92]. There are many different kinds of EDXRF and TRXRF device on the market and technical improvements are frequently announced. Thus, in our opinion, obtaining the I level in a needle biopsy of thyroid nodule, using EDXRF, is a fast, reliable and very informative diagnostic tool that can be successfully used as an additional test for diagnoses of thyroid malignancy.

5. CONCLUSION

In this work, ChEs analysis was carried out in the tissue samples of NT and thyroid with TBN and TMN using three nuclear analytical methods – EDXRF, INAA-SLR, and INAA-LLR. It was shown that the combination of these methods is an adequate analytical tool for the non-destructive determination of nineteen ChEs (Ag, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Sr, and Zn) contents in the tissue samples of human thyroid, including needle-biopsy material. It was observed that in TMN tissue the mean mass fraction of I was lower while the mean mass fractions of K, Mg, and Rb were higher than in both NT and TBN groups of samples. It was demonstrated that I content in nodular tissue is the most informative parameter for the diagnosis of thyroid malignancy. It was found that “Sensitivity”, “Specificity” and “Accuracy” of TMN identification using the I level in the needle biopsy of affected thyroid tissue was significantly higher than that using US examination and

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

cytological test of fine needle aspiration biopsy. It was concluded that determination of the I level in a needle biopsy of TNs, using non-destructive instrumental analytical method, is a fast, reliable, and very informative diagnostic tool that can be successfully used as an additional test of thyroid malignancy identification.

Declaration of Conflicting Interests

The author has not declared any conflict of interests.

Funding

The author received no financial support for this study and for publication of this article.

Acknowledgements

The author is extremely grateful to Profs. B.M. Vtyurin and V.S. Medvedev, Medical Radiological Research Center, Obninsk, as well as to Dr. Yu. Choporov, former Head of the Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples.

REFERENCES

1. Fresilli D, David E, Pacini P, Gaudio GD, Dolcetti V, Lucarelli GT, Di Leo N, Bellini MI, D'Andrea V, Sorrenti S, Mascagni D, Biffoni M, Durante C, Grani G, De Vincentis G, Cantisani V. Thyroid Nodule Characterization: How to Assess the Malignancy Risk. Update of the Literature. *Diagnostics (Basel)* 2021;11(8):1374.
2. Jin Z, Zhu Y, Zhang S, Xie F, Zhang M, Zhang Y, Tian X, Zhang J, Luo Y, Cao J. 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* 2020; 26: e918452.
3. Trimboli P, Castellana M, Piccardo A, Romanelli F, Grani G, Giovanella L, Durante C. The ultrasound risk stratification systems for thyroid nodule have been evaluated against papillary carcinoma. A meta-analysis. *Rev Endocr Metab Disord* 2021;22(2):453-460.
4. Patel SG, Carty SE, Lee AJ. Molecular testing for thyroid nodules including its interpretation and use in clinical practice. *Ann Surg Oncol* 2021;28(13):8884-8891.
5. Silaghi CA, Lozovanu V, Georgescu CE, Georgescu RD, Susman S, Năsui BA, Dobrea A, Silaghi H. Thyroseq v3, Afirma GSC, and microRNA Panels versus previous molecular tests in the preoperative diagnosis of indeterminate thyroid nodules: a systematic review and meta-analysis. *Front Endocrinol (Lausanne)* 2021;12:649522.
6. Zaichick V. Iodine excess and thyroid cancer. *J Trace Elem Exp Med* 1998;11(4):508-509.
7. Zaichick V, Iljina T. Dietary iodine supplementation effect on the rat thyroid ¹³¹I blastomogenic action. In: *Die Bedeutung der Mengen- und Spurenelemente*. 18. Arbeitstagung. Jena: Friedrich-Schiller-Universität; 1998. p. 294-306.
8. Kim K, Cho SW, Park YJ, Lee KE, Lee D-W, Park SK. Association between iodine intake, thyroid function, and papillary thyroid cancer: A case-control study. *Endocrinol Metab (Seoul)* 2021;36(4):790-799.
9. Stojšavljević A, Rovčanin B, Krstić D, Borković-Mitić S, Paunović I, Diklić A, Gavrović-Jankulović M, Manojlović D. 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* 2019.
10. Fahim YA, Sharaf NE, Hasani IW, Ragab EA, Abdelhakim HK. 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, Wang C, Jiang J, Liu X, Wei W, Peng J, Liu S, Li Y, Xu N, Zhou D, Zhang Q, Zhang J. A case-control study on the association of mineral elements exposure and thyroid tumor and goiter. *Ecotoxicol Environ Saf* 2021;208:111615.

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

12. Zaichick V. Medical elementology as a new scientific discipline. *J Radioanal Nucl Chem* 2006;269:303-309.
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* 2017;7:115–119.
14. Beyersmann D, Hartwig A. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. *Arch Toxicol* 2008;82(8):493-512.
15. Martinez-Zamudio R, Ha HC. Environmental epigenetics in metal exposure. *Epigenetics* 2011;6(7):820-827.
16. Zaichik V, Raibukhin YuS, Melnik AD, Cherkashin VI. Neutron-activation analysis in the study of the behavior of iodine in the organism. *Med Radiol (Mosk)* 1970;15(1):33-36.
17. Zaichik V, Matveenko EG, Vtiurin BM, Medvedev VS. Intrathyroid iodine in the diagnosis of thyroid cancer. *Vopr Onkol* 1982;28(3):18-24.
18. Zaichick V, Tsyb AF, Vtyurin BM. Trace elements and thyroid cancer. *Analyst* 1995;120(3):817-821.
19. Zaichick V, Choporov YuYa. Determination of the natural level of human intra-thyroid iodine by instrumental neutron activation analysis. *J Radioanal Nucl Chem* 1996;207(1):153-161.
20. Zaichick V. *In vivo* and *in vitro* application of energy-dispersive XRF in clinical investigations: experience and the future. *J Trace Elem Exp Med* 1998;11(4):509-510.
21. Zaichick V, Zaichick S. Energy-dispersive X-ray fluorescence of iodine in thyroid puncture biopsy specimens. *J Trace Microprobe Tech* 1999;17(2):219-232.
22. Zaichick V. Relevance of, and potentiality for *in vivo* intrathyroidal iodine determination. *Ann N Y Acad Sci* 2000;904:630-632.
23. Zaichick V, Zaichick S. Normal human intrathyroidal iodine. *Sci Total Environ* 1997;206(1):39-56.
24. Zaichick V. 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; 1999. p.114-119.
25. Zaichick V, Zaichick S. Age-related changes of some trace element contents in intact thyroid of females investigated by energy dispersive X-ray fluorescent analysis. *Trends Geriatr Healthc* 2017;1(1):31-38.
26. Zaichick V, Zaichick S. Age-related changes of some trace element contents in intact thyroid of males investigated by energy dispersive X-ray fluorescent analysis. *MOJ Gerontol Ger* 2017;1(5):00028.
27. Zaichick V, Zaichick S. 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* 2017;1:5.1.
28. Zaichick V, Zaichick S. 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* 2017;1(1):1002.
29. Zaichick V, Zaichick S. 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. 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* 2017;4(4):555644.
31. Zaichick V, Zaichick S. Effect of age on chemical element contents in female thyroid investigated by some nuclear analytical methods. *MicroMedicine* 2018;6(1):47-61.
32. Zaichick V, Zaichick S. Neutron activation and X-ray fluorescent analysis in study of association between age and chemical element

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

- contents in thyroid of males. *Op Acc J Bio Eng Bio Sci* 2018;2(4):202-212.
33. Zaichick V, Zaichick S. 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* 2018;3(1):1-10.
 34. Zaichick V, Zaichick S. Association between age and twenty chemical element contents in intact thyroid of males. *SM Gerontol Geriatr Res* 2018;2(1):1014.
 35. Zaichick V, Zaichick S. Associations between age and 50 trace element contents and relationships in intact thyroid of males. *Aging Clin Exp Res* 2018;30(9):1059–1070.
 36. Zaichick V, Zaichick S. Possible role of inadequate quantities of intra-thyroidal bromine, rubidium and zinc in the etiology of female subclinical hypothyroidism. *EC Gynaecology* 2018;7(3):107-115.
 37. Zaichick V, Zaichick S. 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* 2018;1(3):IGWHC.MS.ID.000113.
 38. Zaichick V, Zaichick S. Possible role of inadequate quantities of intra-thyroidal cobalt, rubidium and zinc in the etiology of female subclinical hypothyroidism. *Womens Health Sci J* 2018;2(1):000108.
 39. Zaichick V, Zaichick S. 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* 2018;2(4):340-355.
 40. Zaichick V, Zaichick S. Investigation of association between the high risk of female subclinical hypothyroidism and inadequate quantities of twenty intra-thyroidal chemical elements. *Clin Res: Gynecol Obstet* 2018;1(1):1-18.
 41. Zaichick V, Zaichick S. 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* 2018;2(9):23-37.
 42. Zaichick V. Comparison between trace element contents in macro and micro follicular colloid goiter using energy dispersive X-ray fluorescent analysis. *International Journal of Bioprocess & Biotechnological Advancements* 2021;7(5):399-406.
 43. Zaichick V. Trace element contents in thyroid of patients with diagnosed nodular goiter determined by energy dispersive X-ray fluorescent analysis. *Applied Medical Research* 2021;8(2):1-9.
 44. Zaichick V. Evaluation of trace element in thyroid adenomas using energy dispersive X-ray fluorescent analysis. *Journal of Nanosciences Research & Reports* 2021;3(4):1-7.
 45. Zaichick V. Evaluation of thyroid trace element in Hashimoto's thyroiditis using method of X-ray fluorescence. *International Journal of Integrated Medical Research* 2021;8(4):1-9.
 46. Zaichick V. Evaluation of trace elements in Riedel's Struma using energy dispersive X-ray fluorescence analysis. *International Journal of Radiology Sciences* 2021;3(1):30-34.
 47. Zaichick V, Zaichick S. Trace element contents in thyroid cancer investigated by energy dispersive X-Ray fluorescent analysis. [American Journal of Cancer Research and Reviews](#) 2018;2(5):1-11.
 48. Zaichick V. Content of copper, iron, iodine, rubidium, strontium and zinc in thyroid benign nodules and tissue adjacent to nodules. *International Journal of Medical and Public Health Research and Review* 2021;1(1):30-42.
 49. Zaichick V, Zaichick S. Instrumental effect on the contamination of biomedical samples in

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

- the course of sampling. *The Journal of Analytical Chemistry* 1996;51(12):1200-1205.
50. Zaichick V, Zaichick S. A search for losses of chemical elements during freeze-drying of biological materials. *J Radioanal Nucl Chem* 1997;218(2):249-253.
51. Zaichick V. Applications of synthetic reference materials in the medical Radiological Research Centre. *Fresenius J Anal Chem* 1995;352:219-223.
52. Zaichick V, Zaichick S. INAA application in the assessment of chemical element mass fractions in adult and geriatric prostate glands. *Appl Radiat Isot* 2014;90:62-73.
53. Handl J, Pfau A, Huth FW. Measurements of ¹²⁹I in human and bovine thyroids in Europe-transfer of ¹²⁹I into the food chain. *Health Phys* 1990;58(5):609-618.
54. Aeschimann S, Buergi U, Wagner HE, Kaempf J, Lauber K, Studer H. Low intrathyroidal iodine concentration in non-endemic human goiters: a consequence rather than a cause of autonomous goiter growth. *J Endocrinol* 1994; 140(1):156-164.
55. Boulyga, S.F., Zhuk, I.V., Lomonosova, E.M., Kanash, N.V., Bazhanova, N.N. Determination of microelements in thyroids of the inhabitants of Belarus by neutron activation analysis using the k₀-method. *J Radioanal Nucl Chem* 1997;222(1-2):11-14
56. Boulyga SF, Petri H, Zhuk IV, Kanash NV, Malenchenko AF. Neutron-activation analysis of trace elements in thyroids. *J Radioanal Nucl Chem* 1999;242(2):335-340.
57. Reddy SB, Charles MJ, Kumar MR, Reddy BS, Anjaneyulu Ch, Raju GJN, Sundareswar B, Vijayan V. 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*. 2002;196(3-4):333-339.
58. Wang J, Chen R, Zhu H. Study in China on ingestion and organs content of trace elements of importance in radiological protection. *Food and Nutrition Bulletin* 2002;23(3 Suppl):217-221
59. Murillo M, Carrion N, Quintana M, Sanabria G, Rios M, Duarte L, Ablan F. Determination of selenium and iodine in human thyroids. *J Trace Elem Med Biol* 2005;19:23-27.
60. Hansson M, Grunditz T, Isaksson M, Jansson S, Lausmaa J, Mölne J, Berg G. Iodine content and distribution in extratumoral and tumor thyroid tissue analyzed with X-ray fluorescence and time-of-flight secondary ion mass spectrometry. *Thyroid* 2008;18(11):1215-1220.
61. Zabala J, Carrion N, Murillo M, Quintana M, Chirinos J, Seijas N, Duarte L, Brätter P. Determination of normal human intrathyroidal iodine in Caracas population. *J Trace Elem Med Bio* 2009;23(1):9-14.
62. Zhu H, Wang N, Zhang Y, Wu Q, Chen R, Gao J, Chang P, Liu Q, Fan T, Li J, Wang J, Wang J. Element contents in organs and tissues of Chinese adult men. *Health Phys* 2010;98(1):61-73.
63. Błazewicz A, Orlicz-Szczesna G, Szczesny P, Prystupa A, Grzywa-Celinska A, Trojnar M. 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* 2011;879:573-578.
64. Zaichick V, Zaichick S. Variation in Selected Chemical Element Contents Associated with Malignant Tumors of Human Thyroid Gland. *Cancer Studies*. 2018a; 2(1):2, pp. 1-12.
65. Zaichick V, Zaichick S. Twenty Chemical Element Contents in Normal and Cancerous Thyroid. *Int J Hematol Blo Dis* 2018b;3(2):1-13
66. Zaichick V, Zaichick S. Levels of chemical element contents in thyroid as potential biomarkers for cancer diagnosis (a preliminary study). *J Cancer Metastasis Treat* 2018c;4:60.
67. Zaichick V. Determination the content of bromine, calcium, chlorine, iodine, potassium, magnesium, manganese, and sodium in the nodular goiter of human thyroid gland using

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

- neutron activation analysis. *Aditum Journal of Clinical and Biomedical Research* 2021a; 3(3): 1-8,
68. Zaichick V. Evaluation of bromine, calcium, chlorine, iodine, potassium, magnesium, manganese, and sodium content in the thyroid adenomas using neutron activation analysis. *J Carcinog Mutagen* 2021b;12:366.
69. Zaichick V. Comparison bromine, calcium, chlorine, iodine, potassium, magnesium, manganese, and sodium contents in normal thyroid and thyroid with Hashimoto's thyroiditis. *J Clin Res Oncol* 2021c;4(1):21-27.
70. Zaichick V. Comparison between bromine, calcium, chlorine, iodine, potassium, magnesium, manganese, and sodium contents in normal thyroid and Riedel's Struma. *Journal of Biotechnology and Bioinformatics Research*. 2021;3(4):1-6
71. Zaichick V. Comparison of twenty chemical element contents in normal thyroid tissue and hypertrophic thyroid tissue. *Universal Journal of Pharmaceutical Research* 2021; 6(4): 32-42
72. Zaichick V. 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* 2021;11(03):242–257
73. Nishita M, Sakurai H, Tezuka U, Kawada J, Koyama M, Takada J. Alteration in manganese and iodide contents in human thyroid tumors; a correlation between the contents of essential trace elements and the states of malignancy. *Clin Chem Acta* 1990;187(2):181-188.
74. Bellisola G, Bratter P, Cinque C, Francia G, Galassini S, Gawlik D, [Negretti de Brätter VE](#), [Azzolina L](#). The TSH-dependent variation of the essential elements iodine, selenium, and zinc within human thyroid tissue. *J Trace Elem Med Biol* 1998;12:177-182.
75. Zaichick V.. Determination of Twenty Chemical Element Contents in Normal and Goitrous Thyroid using X-Ray Fluorescent and Neutron Activation Analysis. *World Journal of Advanced Research and Reviews* 2021; 11(02): 130–146.
76. Zaichick V. Evaluation of Twenty Chemical Element Contents in Thyroid Adenomas using X-Ray Fluorescent and Neutron Activation Analysis. *J Cell Mol Onco* 2021;1(3):007.
77. Zaichick V. 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*. 2021;2(10):1500–1510.
78. Zaichick V. Comparison of Nineteen Chemical Element Contents in Normal Thyroid and Thyroid with Riedel's Struma. *Journal of Medical Research and Health Sciences*. 2021;4(11):1529–1538.
79. Zaichick V. Content of Copper, Iron, Iodine, Rubidium, Strontium and Zinc in Thyroid Benign Nodules and Tissue adjacent to Nodules. *International Journal of Medical and Public Health Research and Review* 2021; 1(1): 30-42.
80. Zaichick V. Contents of Nineteen Chemical Elements in Thyroid Benign Nodules and Tissue adjacent to Nodules investigated using Neutron Activation Analysis and Inductively Coupled Plasma Atomic Emission Spectrometry. *Research and Reviews on Healthcare: Open Access Journal* 2022;7(3):719-727
81. Zaichick V. Content of Copper, Iron, Iodine, Rubidium, Strontium and Zinc in Thyroid Malignant Nodules and Thyroid Tissue adjacent to Nodules. *Journal of Clinical and Diagnostic Pathology* 2022a;1(4):7-17.
82. Zaichick V. Contents of Calcium, Chlorine, Iodine, Potassium, Magnesium, Manganese, and Sodium in Thyroid Malignant Nodules and Thyroid Tissue adjacent to Nodules *J Med Case Rep Rev* 2022b;5(2):1068-1078.
83. Zaichick V. Content of Eleven Trace Elements in Thyroid Malignant Nodules and Thyroid Tissue adjacent to Nodules. *Interventions in*

Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods

Gynaecology and Women Health Care 2022c;5(1):468-476

84. Zaichick V. Contents of Nineteen Chemical Elements in Thyroid Malignant Nodules and Thyroid Tissue adjacent to Nodules investigated using X-Ray Fluorescence and Neutron Activation Analysis. *Journal of Medical Research and Health Sciences* 2022d;5(1):1663-1677.
85. 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* 2022e;7(1):45-56.
86. Katoh Y, Sato T, Yamamoto Y. Determination of multielement concentrations in normal human organs from the Japanese. *Biol Trace Elem Res* 2002;90(1-3):57-70.
87. Schroeder HA, Tipton IH, Nason AP. Trace metals in man: strontium and barium. *J Chron Dis* 1972;25(9):491-517.
88. Genes VS. Simple methods for cybernetic data treatment of diagnostic and physiological studies. Moscow:Nauka; 1967.
89. Zaichick V. 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. Vienna: IAEA; 1997. p.123-133.
90. Zaichick V, Zaichick S. A search for losses of chemical elements during freeze-drying of biological materials. *J Radioanal Nucl Chem* 1997;218(2):249-253.
91. Zaichick V. Losses of chemical elements in biological samples under the dry aching process. *Trace Elements in Medicine* 2004;5(3):17-22.
92. Rossmann M, Zaichick S, Zaichick V. Determination of key chemical elements by energy dispersive X-Ray fluorescence analysis in commercially available infant and toddler

formulas consumed in UK. *Nutr Food Technol Open Access* 2016;2(4):1-7.

How to cite this article: Zaichick V.. Diagnosis of Thyroid Malignancy using Chemical Elements of Nodular Tissue determined by Nuclear Analytical Methods. *Journal of Medical Research and Health Sciences.* 2022;5(3) 1809–1824. <https://doi.org/10.52845/JMRHS/2022-5-3>