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Diagnosis of thyroid malignancy using levels of trace element contents in nodular tissue

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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.
Objective: The aim of present study was to evaluate possibilities of using differences in trace elements (TEs) contents in nodular tissue for diagnosis of thyroid malignancy.
Methods: Contents of thirty two TEs such as silver (Ag), aluminum (Al), boron (B),, beryllium (Be), bismuth (Bi), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), cesium (Cs), iron (Fe), gallium (Ga), mercury (Hg), iodine (I), lanthanum (La), lithium (Li), manganese (Mn), molybdenum (Mo), neodymium (Nd), nickel (Ni), lead (Pb), praseodymium (Pr), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), samarium (Sm), tin (Sn), thallium (Tl), uranium (U), yttrium (Y), 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 instrumental neutron activation analysis with destructive method such as inductively coupled plasma mass spectrometry.
Results: It was observed that in TMN tissue the mean mass fractions of I and Sc were lower while the mean mass fractions of Mo 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 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; trace elements; neutron activation analysis; inductively coupled plasma mass spectrometry

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]. 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” [2]. Thus, to improve diagnostic accuracy of TMN, new technologies have to be developed for clinical applications.
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]. Among these factors a disturbance of evolutionary stable input of many trace elements (TEs) in human body after industrial revolution plays a significant role in etiology of TNs. Besides iodine, many other TEs have also essential physiological role and involved in thyroid functions. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of TEs depend on tissue-specific need or tolerance, respectively [4]. Excessive accumulation or an imbalance of the TEs

may disturb the cell functions and may result in cellular proliferation, degeneration, death, benign or malignant transformation [4, 5].

In our previous studies the complex of *in vivo* and *in vitro* nuclear analytical and related methods was developed and used for the investigation of iodine and other TEs contents in the normal and pathological thyroid [6-8]. Iodine level in the normal thyroid was investigated in relation to age, gender and some non-thyroidal diseases [9]. After that, variations of many TEs content with age in the thyroid of males and females were studied and age- and gender-dependence of some TEs was observed [10-18].

The present study had two aims. The main objective was to assess the silver (Ag), aluminum (Al), boron (B),, beryllium (Be), bismuth (Bi), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), cesium (Cs), iron (Fe), gallium (Ga), mercury (Hg), iodine (I), lanthanum (La), lithium (Li), manganese (Mn), molybdenum (Mo), neodymium (Nd), nickel (Ni), lead (Pb), praseodymium (Pr), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), samarium (Sm), tin (Sn), thallium (Tl), uranium (U), yttrium (Y), and zinc (Zn) contents in "normal" thyroid (NT) as well as in nodular tissue of patients who had either TBN or TMN using a combination of non-destructive instrumental neutron activation analysis with high resolution spectrometry of short- and long-lived radionuclides (INAA-SLR and INAA-LLR, respectively) and destructive method such as inductively coupled plasma mass spectrometry (ICP-MS). The second aim was to evaluate TEs content to aid diagnosis of thyroid malignancy.

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 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±SD was 44±11 years, range 22-64) and from TMN (n=41, mean age M±SD was 46±15 years, range 16-75) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre (MRRC), Obninsk. Thick-needle puncture biopsy of suspicious nodules of the thyroid was performed for every patient, to permit morphological study of thyroid tissue at these sites and to estimate their TEs contents. In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. Histological conclusions for TBN were: 46 colloid 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 TEs 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 TEs of stainless steel [19]. One was used for morphological study while the other was intended for TEs analysis. After the samples intended for TEs analysis were weighed, they were freeze-dried and homogenized [20].

To determine the contents of the TEs by comparison with known data for standard, aliquots of commercial, chemically pure compounds and synthetic reference materials were used [21]. Ten sub-samples of certified reference material (CRM) IAEA H-4 (animal muscle) and five sub-samples of CRM of the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results.

The content of I 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 nuclear reaction, radionuclide, gamma-energies, spectrometric unit, sample preparation, and the quality control of results were presented in our earlier publications concerning the INAA-SLR of I contents in human thyroid [6, 10, 11].

A vertical channel of the same nuclear reactor was applied to determine the content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-LLR. Details of used nuclear reactions, radionuclides, gamma-energies, spectrometric unit, sample preparation and procedure of measurement were presented in our earlier publications concerning the INAA-LLR of TEs contents in human thyroid [12, 13].

After non-destructive INAA-LLR investigation the thyroid samples were used for ICP-MS. The samples were decomposed in autoclaves and aliquots of solutions were used to determine the Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr mass fractions by ICP-MS using an ICP-MS Thermo-Fisher "X-7" Spectrometer (Thermo Electron, USA). Information detailing with the NAA-LLR and ICP-MS methods used and other details of the analysis were presented in our earlier publications concerning TE contents in human thyroid [22].

All samples for TEs analysis were prepared in duplicate, and mean values of TEs contents were used in final calculation. Mean values of TEs contents were used in final calculation for the Ag, Co, Cr, Hg, Rb, Sb, Se, and

Zn mass fractions measured by both INAA-LLR and ICP-MS 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 TEs 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.

Results

Table 1 depicts certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, range) of the Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hg, I, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, 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, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hg, I, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, 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, Mo, Rb, and Sc mass fraction in all samples of NT, TBN, and TMN group.

Parameters of the sensitivity, specificity and accuracy (M±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.

The comparison of our results with published data (from 1990 year) for I mass fraction in NT [10, 11, 14-18, 23-42], TBN [24, 26, 27, 32, 33, 37-50], and TMN [24, 26, 27, 30, 34-36, 43, 44, 51-55] 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%) [56] and ash (4.16% on dry mass basis) [57] contents in thyroid of adults.

Discussion

As was shown before [6, 10-13, 22] good agreement of the Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hg, I, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, and Zn contents in CRM IAEA H-4, INCT-SBF-4, INCT-TL-1, and INCT-MPH-2 samples determined by both INAA-SLR and ICP-MS methods 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 fraction of I and Sc are significantly lower while the mass fraction of Mo and Rb are higher than in both NT and TBN groups of samples. 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) 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:

Sensitivity = {correct positive test (CPT)/ [CPT + false negative test (FNT)]} ×100% = 84±6%;

Specificity = {correct negative test (CNT)/ [CNT + false positive test (FPT)]} ×100% = 96±2%;

Accuracy = [(CPT+CNT)/ (CPT+FNT+CNT+FPT)] ×100% = 94±2%.

The number of people examined was taken into account for calculation of confidence intervals [58]. 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).

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 [1, 2]. 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]. 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" [1, 2].

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 [59].

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 TEs contents in a microprobe of a human body tissues and fluids within a few minutes [60]. 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 TEs determination [60]. 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.

Table 1: Basic statistical parameters of Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Ti, U, Y, 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±SD (SEM)	Range	Mean±SD (SEM)	Range	Mean±SD (SEM)	Range
Ag	0.013±0.011(0.001)	0.002-0.08	0.192±0.199(0.028)	0.002-0.842	0.139±0.141(0.028)	0.007-0.54
Al	10.5±13.4(1.8)	0.80-69.3	27.3±23.6(4.2)	6.6-95.1	33.0±25.5(7.1)	4.5-96.5
B	0.476±0.434(0.058)	0.200-2.3	4.65±15.0(2.7)	0.81-85.2	2.21±1.89(0.52)	1.0-5.6
Be	0.0005±0.0006 (0.0001)	0.0001-0.003	0.0009±0.0011 (0.0002)	0.0002-0.006	0.0005±0.0001 (0.00004)	0.0002-0.0007
Bi	0.0072±0.0161 (0.0022)	0.0003-0.100	0.071±0.085 (0.016)	0.004-0.42	0.067±0.083 (0.023)	0.005-0.335
Cd	2.08±2.05(0.27)	0.0110-8.26	1.55±1.68(0.30)	0.13-6.4	1.13±1.82 (0.49)	0.03-6.83
Ce	0.0080±0.0080 (0.0011)	0.0010-0.035	0.018±0.017(0.003)	0.003-0.07	0.0277±0.0275 (0.0080)	0.005-0.087
Co	0.0390±0.0276(0.0031)	0.0100-0.140	0.058±0.032(0.005)	0.015-0.16	0.0499±0.0292 (0.0050)	0.004-0.143
Cr	0.495±0.261(0.031)	0.130-1.30	1.17±1.19(0.17)	0.075-7.3	1.85±1.81(0.15)	0.04-3.50
Cs	0.0245±0.0166 (0.0022)	0.0022-0.092	0.032±0.047(0.009)	0.008-0.21	0.0298±0.0287 (0.0090)	0.007-0.112
Fe	222.8±89.5(9.6)	52.0-474	430±566(67)	52-2734	255±168(27)	61-880
Ga	0.0316±0.0156 (0.0021)	0.010-0.081	0.021±0.008(0.002)	0.010-0.034	0.034±0.011 (0.003)	0.020-0.064
Hg	0.0543±0.0373 (0.0043)	0.0070-0.15	1.15±1.04(0.14)	0.1-5.2	0.922±0.83(0.15)	0.07-3.75
I	1841±1027(107)	114-5061	1086±1219(139)	29.0-8260	71.8±62.0(10.1)	2.00-261
La	0.0048±0.0046 (0.0006)	0.0004-0.022	0.009±0.009(0.002)	0.002-0.036	0.013±0.012(0.004)	0.004-0.044
Li	0.0208±0.0155 (0.0022)	0.0015-0.098	0.030±0.015(0.003)	0.007-0.068	0.032±0.031(0.009)	0.008-0.111
Mn	1.28±0.56(0.07)	0.470-4.04	1.81±1.41(0.21)	0.1-6.1	2.01±1.34(0.29)	0.10-5.95
Mo	0.0836±0.0470 (0.0062)	0.0104-0.30	0.19±0.12(0.02)	0.05-0.63	0.29±0.11(0.03)	0.09-0.53
Nd	0.0041±0.0034 (0.0004)	0.0002-0.017	0.013±0.007(0.002)	0.003-0.033	0.016±0.014(0.005)	0.003-0.041
Ni	0.449±0.344(0.046)	0.0740-	2.89±2.52(0.47)	0.1-10.4	4.38±2.24(0.65)	0.27-7.30

		1.80				
Pb	0.233±0.246(0.033)	0.0230-1.60	1.31±2.27(0.41)	0.1-9.3	1.14±1.16(0.33)	0.24-4.44
Pr	0.0011±0.0009(0.0001)	0.0001-0.004	0.004±0.003(0.001)	0.001-0.013	0.008±0.013(0.004)	0.001-0.046
Rb	7.54±3.65(0.39)	1.21-22.6	9.50±4.23(0.50)	2.5-22.1	12.7±4.9(0.8)	5.1-27.4
Sb	0.0947±0.0692(0.0075)	0.0047-0.31	0.12±0.11(0.01)	0.002-0.47	0.107±0.075(0.014)	0.016-0.334
Sc	0.0268±0.0329(0.0060)	0.0002-0.09	0.025±0.038(0.006)	0.0002-0.15	0.008±0.013(0.002)	0.0002-0.056
Se	2.22±1.24(0.14)	0.320-5.80	3.20±2.92(0.39)	0.7-13.8	2.04±1.06(0.19)	0.14-4.80
Sm	0.0005±0.0005(0.0001)	0.0001-0.002	0.0017±0.0018(0.0003)	0.0004-0.008	0.0019±0.0017(0.0005)	0.0005-0.0067
Sn	0.0777±0.0677(0.0091)	0.009-0.26	0.052±0.040(0.007)	0.014-0.172	0.070±0.049(0.014)	0.014-0.182
Tl	0.0009±0.0005 (0.0001)	0.0001-0.003	0.0019±0.0011(0.0002)	0.0005-0.0054	0.003±0.002(0.001)	0.0006-0.007
U	0.0004±0.0004 (0.0001)	0.0001-0.003	0.0012±0.0006(0.0002)	0.0004-0.0024	0.005±0.011(0.004)	0.0006-0.033
Y	0.0026±0.0023(0.0003)	0.0010-0.011	0.011±0.010(0.003)	0.003-0.036	0.012±0.012(0.004)	0.002-0.034
Zn	94.8±39.6(4.2)	7.10-215	117.7±48.7(5.8)	47-264	96.9±80.0(12.6)	28.7-375

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 trace elements 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	***p* t-test**	***p* U-test**	**Ratio TMN/NT**	***p* t-test**	***p* U-test**	**Ratio TMN/TBN**	***p* t-test**	***p* U-test**
Ag	14.4	<0.000001	≤0.01	10.5	0.00013	≤0.01	0.72	0.178	>0.05
Al	2.60	0.00059	≤0.01	3.14	0.0083	≤0.01	1.21	0.497	>0.05
B	9.77	0.133	>0.05	4.64	0.0062	≤0.01	0.48	0.381	>0.05
Be	1.73	0.093	>0.05	0.90	0.589	>0.05	0.52	0.048	≤0.01
Bi	9.81	0.00050	≤0.01	9.31	0.024	≤0.01	0.95	0.893	>0.05
Cd	0.75	0.192	>0.05	0.54	0.103	>0.05	0.73	0.472	>0.05
Ce	2.26	0.0064	≤0.01	3.46	0.025	≤0.01	1.53	0.267	>0.05
Co	1.48	0.00093	≤0.01	1.28	0.082	>0.05	0.87	0.276	>0.05
Cr	2.36	0.00023	≤0.01	3.74	0.026	≤0.01	1.58	0.150	>0.05
Cs	1.31	0.423	>0.05	1.22	0.573	>0.05	0.93	0.857	>0.05
Fe	1.93	0.0031	≤0.01	1.14	0.270	>0.05	0.59	0.018	≤0.01
Ga	0.67	0.00038	≤0.01	1.08	0.519	>0.05	1.62	0.0034	≤0.01
Hg	21.2	<0.000001	≤0.01	16.9	<0.000001	≤0.01	0.80	0.248	>0.05
I	0.59	0.00003	≤0.01	0.039	<0.00001	≤0.01	0.0066	<0.00001	≤0.01
La	1.98	0.017	≤0.01	2.82	0.070	>0.05	1.43	0.385	>0.05
Li	1.42	0.018	≤0.01	1.51	0.265	>0.05	1.07	0.832	>0.05
Mn	1.41	0.022	≤0.01	1.57	0.025	≤0.01	1.11	0.589	>0.05
Mo	2.31	0.000017	≤0.01	3.49	0.000017	≤0.01	1.51	0.015	≤0.01
Nd	3.27	0.000020	≤0.01	3.80	0.056	>0.05	1.16	0.692	>0.05
Ni	6.44	0.000016	≤0.01	9.76	0.000079	≤0.01	1.52	0.074	≤0.05
Pb	5.62	0.013	≤0.01	4.89	0.020	≤0.01	0.87	0.754	>0.05
Pr	3.64	0.00019	≤0.01	7.29	0.115	>0.05	2.01	0.343	>0.05
Rb	1.26	0.0025	≤0.01	1.68	<0.000001	≤0.01	1.33	0.00093	≤0.01
Sb	1.28	0.122	>0.05	1.13	0.388	>0.05	0.88	0.556	>0.05
Sc	0.89	0.717	>0.05	0.29	0.0053	≤0.01	0.32	0.0094	≤0.01
Se	1.44	0.020	≤0.01	0.92	0.457	>0.05	0.64	0.0097	≤0.01
Sm	3.37	0.00079	≤0.01	3,83	0.012	≤0.01	1.13	0.696	>0.05
Sn	0.66	0.027	≤0.01	0.90	0.627	>0.05	1.35	0.253	>0.05
Tl	2.04	0.000065	≤0.01	3.29	0.0020	≤0.01	1.62	0.062	≤0.05
U	2.62	0.0036	≤0.01	11.6	0.270	>0.05	4.43	0.345	>0.05
Y	4.23	0.0044	≤0.01	4.73	0.071	>0.05	1.12	0.808	>0.05
Zn	1.24	0.0018	≤0.01	1.02	0.877	>0.05	0.82	0.141	>0.05

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

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

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 [23]	Chem	39	21-86	-	1276±664	-
Aeschimann *et al*.1994 [24]	Chem	1	-	AD	2028	-
Boulyga *et al*. 1997 [25]	NAA	29	-	D, A	1778±381	-
	NAA	10	-	D, A	1905±635	-
Boulyga *et al*. 1999 [26]	NAA	12	-	D, A	-	800-2950
Reddy *et al*. 2002 [27]	PIXE	4	-	D, Press	916±88	-
Wang *et al*. 2002 [28]	-	21	Adult	-	2712±800	-
Murillo *et al*. 2005 [29]	Color	5	30-43	AD	948-3356	948-3356
Hansson *et al*. 2008 [30]	EDXRF	10	57-80	Intact	2400	1200-4800
Zabala *et al*. 2009 [31]	SFI	50	17-60	AD	5772±2708	1676-13720
Zhu *et al*. 2010 [32]	ICPMS	50	20-60	AD	2648	964-4760
Błazewicz *et al*. 2011 [33]	IC	50	M=25	Fixed	601±192	624-4020
				Frozen	623±187	840 -4000
Zaichick *et al*. 2017 [10]	NAA	72	2-80	Intact	1786±940	220-4205
Zaichick *et al*. 2017 [11]	NAA	33	3.5-87	Intact	1956±1199	114-5061
Zaichick *et al*. 2018 [14]	EDXRF, NAA	72	2-80	Intact	1786±940	220-4205
Zaichick *et al*. 2018 [15]	EDXRF, NAA	33	3.5-87	Intact	1956±1199	114-5061
Zaichick *et al*. 2018 [16]	NAA, ICPAES	33	3.5-87	Intact	1956±1199	114-5061
Zaichick *et al*. 2018 [17]	NAA, ICPAES	72	2-80	Intact	1786±940	220-4205
Zaichick *et al*. 2018 [18]	NAA	105	2-80	Intact	1841±1027	114-5061
Zaichick *et al*. 2018 [34]	NAA	105	44±21	Intact	1841±1027	114-5061
Zaichick *et al*. 2018 [35]	NAA	105	2-80	Intact	1841±1027	114-5061
Zaichick *et al*. 2018 [36]	NAA	105	44±21	Intact	1841±1027	114-5061
Zaichick, 2021 [37]	NAA	105	2-87	Intact	1841±1027	114-5061
Zaichick, 2021 [38]	NAA	105	44±21	Intact	1841±1027	114-5061
Zaichick, 2021 [39]	NAA	105	2-87	Intact	1841±1027	114-5061
Zaichick, 2021 [40]	NAA	105	44±21	Intact	1841±1027	114-5061
Zaichick, 2021 [41]	NAA, ICPAES	105	2-87	Intact	1841±1027	114-5061
Zaichick, 2021 [42]	NAA, ICPAES	105	44±21	Intact	1841±1027	114-5061
Median of means		1841 mg/kg dry tissue				
Range of means (M_{min} - M_{max}),		(601 – 5772) mg/kg dry tissue				
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 [43]	NAA	14	28-71	Washed	396±74	66-1028
	NAA	7	18-74	Washed	115±40	21-344
Aeschimann *et al*.1994 [24]	Chem	11	-	AD	516	92-3548
Bellisola *et al*. 1998 [44]	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 [26]	NAA	19	-	Washed -	-	100-4050
Reddy *et al*. 2002 [27]	PIXE	4	-	D, Press	888±88	-

Zhu *et al*. 2010 [32]	ICPMS	50	20-60	AD	2648	964-4760
Błazewicz *et al*. 2011 [33]	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 [37]	NAA	46	30-64	Intact	1141±931	29-3715
Zaichick, 2021 [38]	NAA	19	41±11	Intact	961±1013	131-3906
Zaichick, 2021 [39]	NAA	8	40±10	Intact	951±630	83-1787
Zaichick, 2021 [40]	NAA	6	39±9	Intact	276±283	85-824
Zaichick, 2021 [41]	NAA, ICPAES	46	30-64	Intact	1141±931	29-3715
Zaichick, 2021 [42]	NAA, ICPAES	19	41±11	Intact	961±1013	131-3906
Zaichick, 2021 [45]	EDXRF, NAA	46	30-64	Intact	1144±943	29-3715
Zaichick, 2021 [46]	EDXRF, NAA	19	22-55	Intact	962±1013	131-3906
Zaichick, 2021 [47]	EDXRF, NAA	8	34-55	Intact	951±630	83-1787
Zaichick, 2021 [48]	NAA	6	34-50	Intact	276±283	85-824
Zaichick, 2022 [49]	EDXRF	79	22-64	Intact	1107±1358	47-8260
Zaichick, 2022 [50]	NAA, ICPAES	79	22-64	Intact	1086±1219	29-8260
Median of means		920 mg/kg dry tissue				
Range of means (M_{min} - M_{max}),		(77 – 2648) mg/kg dry tissue				
Ratio M_{max}/M_{min}		34.4				
All references		20				

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

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 [43]	NAA	8	21-67	Washed	≤23±10	<DL-67
Aeschimann *et al*. 1994 [24]	Chem	4	-	AD	40	16-140
Bellisola *et al*. 1998 [44]	NAA	12	17-82	Washed	200±210	6 -.430
Boulyga *et al*. 1999 [26]	NAA	19	-	-	-	32-900
Reddy *et al*. 2002 [27]	PIXE	4	-	D, Press	<30	-
Hansson *et al*. 2008 [30]	EDXRF	7	21-58	Intact	<400	-
Zaichick *et al*. 2018 [34]	NAA	41	16-75	Intact	71.8±62	2-261
Zaichick *et al*. 2018 [35]	EDXRF, NAA	41	46±15	Intact	71.8±62	2-261
Zaichick *et al*. 2018 [36]	NAA, ICPAES	41	16-75	Intact	71.8±62	2-261
Zaichick, 2022 [51]	EDXRF	41	16-75	Intact	71.6±72.5	2-341
Zaichick, 2022 [52]	NAA	41	16-75	Intact	71.8±62	2-261
Zaichick, 2022 [53]	NAA	41	16-75	Intact	71.8±62	2-261
Zaichick, 2022 [54]	EDXRF, NAA	41	16-75	Intact	71.8±62	2-261
Zaichick, 2022 [55]	NAA, ICPAES	41	16-75	Intact	71.8±62	2-261
Median of means		71.8 mg/kg dry tissue				
Range of means (M_{min} - M_{max}),		(23 – 400) mg/kg dry tissue				
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

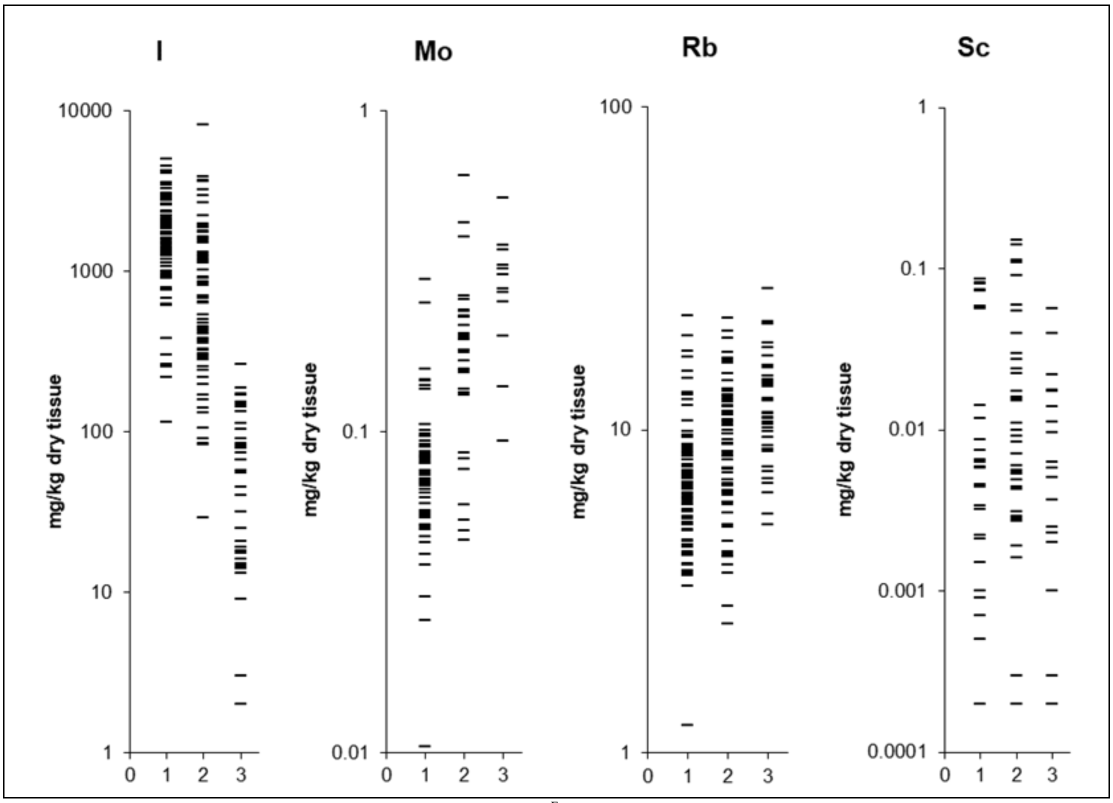

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Fig 1: Individual data sets for I, Mo, Rb, and Sc mass fractions in samples of normal thyroid (1), thyroid benign nodules (2) and thyroid malignant nodules (3).

Conclusion

In this work, TEs analysis was carried out in the tissue samples of NT and thyroid with TBN and TMN using a combination of non-destructive instrumental neutron activation analysis with destructive method such as inductively coupled plasma mass spectrometry. It was shown that this combination is an adequate analytical tool for the non-destructive determination of thirty two TEs such as Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hg, I, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, and Zn in the tissue samples of human thyroid, including needle-biopsy material. It was observed that in TMN tissue the mean mass fractions of I and Sc were lower while the mean mass fractions of Mo 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 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.

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Conflict of Interest

The author has not declared any conflict of interests.

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