**Reviewer’s Comments**

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**Diagnosis of Thyroid Malignancy using Trace Elements of Nodular Tissue determined by X-Ray Fluorescence Analysis**

**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. The aim of present study was (is) to evaluate possibilities of using differences in trace elements (TEs) contents in nodular tissue for diagnosis of thyroid malignancy.

**Method**s: Contents of TEs such as bromine (Br), copper (Cu), iron (Fe), iodine (I), rubidium (Rb), 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 energy-dispersive X-ray fluorescent analysis.

**Results**: It was observed that in TMN tissue the mean mass fractions of I and Zn were lower while the mean mass fraction of Rb was higher than in NT and TBN tissue. It was demonstrated that I content is 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 in which they were 87±5%, 96±2% and 94±2% respectively.

**Conclusions**: It was concluded that study of the I level in a needle biopsy of TNs, obtained by using (energy-dispersive X-ray fluorescent analysis) EDXRF, 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; Energy-dispersive X-ray fluorescent analysis

**Introduction**

Nodules are a common thyroid lesion, particularly in women. Depending on the method of examination and general population, thyroid nodules (TNs) have an incidence of 19–68% [[1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6977643/#b1-medscimonit-26-e918452)]. In clinical practice, TNs are classified into benign (TBN) and malignant (TMN), and among all TNs approximately 10% are TMN [2]. It is appropriate mention here that the incidence of TMN is increasing rapidly (about 5% each year) worldwide [2]. Surgical treatment is not always necessary for TBN whereas surgical treatment is required in TMN. Thus, differentiated TBN and TMN have a great influence on thyroid therapy.

Ultrasound (US) examination widely use as the primary method for early detection and diagnosis of the TNs. However, there are many similarities in the US characteristics of both TBN and TMN. For misdiagnosis prevention some computer-diagnosis systems based on the analysis of US images were developed, however as usual these systems for the diagnosis of TMN showed accuracy, sensitivity, and specificity nearly 80% [2,3]. Therefore, when US examination shows suspicious signs, an US-guided fine-needle aspiration biopsy is advised. Despite the fine needle aspiration biopsy has remained the diagnostic tool of choice for evaluation of US suspicious thyroid nodules, the differentiation of TMN often remains a diagnostic and clinical challenge since up to 30% of nodules are categorized as cytologically “indeterminate” [4]. Thus, to improve diagnostic accuracy of TMN, new technologies have to be developed for clinical applications. However, a recent systematic review and meta-analysis of molecular tests in the preoperative diagnosis of indeterminate TNs shown that at the current time there is no perfect biochemical, immunological, and genetic biomarkers to discriminate malignancy [5]. Therefore, further improvements of TMN diagnostic accuracy are warranted.

During the last decades it was demonstrated that besides the iodine deficiency and excess many other dietary, environmental, and occupational factors are associated with the TNs incidence [3,9-11]. Among these factors a disturbance of evolutionary stable input of many trace elements (TEs) 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 TEs depend on tissue-specific need or tolerance, respectively [13].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 [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 TEs 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 TEs content with age in the thyroid of males and females were studied and age- and gender-dependence of some TEs was observed [25-41]. Furthermore, a significant difference between some TEs 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 bromine (Br), copper (Cu), iron (Fe), iodine (I), rubidium (Rb), 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 combination of non-destructive 109Cd and 241Am radionuclide-induced energy-dispersive X-ray fluorescent analysis (109Cd-EDXRF and 241Am-EDXRF, respectively). 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 109Cd-EDXRF and 241Am-EDXRF 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 [49]. 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 [50].

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 [51]. Ten subsamples of the Certified Reference Material (CRM) IAEA H-4 (animal muscle) were analyzed to estimate the precision and accuracy of results. The CRM IAEA H-4 subsamples were prepared in the same way as the samples of dry homogenized nodular tissue.

Details of the relevant facility for 109Cd-EDXRF determination of Br, Cu, Fe, Rb, Sr, and Zn contents, methods of analysis and the quality control of results were presented in our earlier publications concerning the 109Cd-EDXRF analysis of human thyroid and prostate tissue [25,26,47,52]. Detailed information on EDXRF determination of I contents with 241Am radionuclide source, including methods of analysis and the quality control of results were presented in our earlier publication concerning the use of 241Am-EDXRF analysis in human thyroid study [21].

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

**Specificity, sensitivity and accuracy analysis**:

**Results**

Table 1 depicts certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, range) of the Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction in thyroid tissue samples of three groups – NT, TBN and TMN.

**Table 1**. Basic statistical parameters of Br, Cu, Fe, I, Rb, 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±SD(SEM) | Range | Mean±SD(SEM) | Range | Mean±SD(SEM) | Range |
| Br | 13.9±12.0(1.3) | 1.4-54.4 | 412±682(98) | 3.20-2628 | 139±203(36) | 6.2-802 |
| Cu | 4.23±1.52(0.18) | 0.50-7.50 | 10.2±9.2(1.7) | 2.90-35.2 | 14.5±9.4(2.6) | 4.00-32.6 |
| Fe | 222±102(11) | 47.1-512 | 345±416(49) | 52,0-2563 | 238±184(30) | 54-893 |
| I | 1618±1041(108) | 110-5150 | 1447±3313(373) | 47.0-28000 | 71.6±72.5(11.6) | 2.00-341 |
| Rb | 9.03±6.17(0.66) | 1.80-42.9 | 8.77±4.49(0.53) | 1.00-20.3 | 12.4±5.00(0.79) | 4.80-27.4 |
| Sr | 4.55±3.22(0.37) | 0.10-13.7 | 4.48±6.84(0.88) | 0.42-32.0 | 6.25±7.83(1.63) | 0.93-30.8 |
| Zn | 112±44.0(4.7) | 6.10-221 | 112.9±51.4(6.1) | 22.0-270 | 84.3±57.4(9.2) | 36.7-277 |

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

The ratios of means and the comparison of mean values of Br, Cu, Fe, I, Rb, 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 Br, I, Rb, and Zn 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.

**Table 2.** Ratio of means and the difference between mean values of Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid (NT) and in thyroid benign (TBN) and malignant (TMN) nodules

|  |  |  |  |
| --- | --- | --- | --- |
| El | TBN and NT | TMN and NT | TMN and TBN |
| RatioTBN /NT  | *p* t-test | *p* U-test | RatioTMN/NT | *p* t-test | *p* U-test | RatioTMN/ TBN | *p* t-test | *p* U-test |
| Br | 29.6 | **0.0002** | **≤0.01** | 10.0 | **0.0015** | **≤0.01** | 0.34 | **0.017** | **≤0.01** |
| Cu | 6.67 | **0.0018** | **≤0.01** | 3.43 | **0.0019** | **≤0.01** | 1.42 | 0.176 | >0.05 |
| Fe | 1.55 | **0.018** | **≤0.01** | 1.07 | 0.610 | >0.05 | 0.69 | 0.069 | >0.05 |
| I | 0.89 | 0.661 | >0.05 | 0.044 | **<0.0001** | **≤0.01** | 0.049 | **0.0004** | **≤0.01** |
| Rb | 0.97 | 0.757 | >0.05 | 1.37 | **0.0013** | **≤0.01** | 1.41 | **0.0002** | **≤0.01** |
| Sr | 0.98 | 0.948 | >0.05 | 1.37 | 0.319 | >0.05 | 1.40 | 0.348 | >0.05 |
| Zn | 1.00 | 0.944 | >0.05 | 0.75 | **0.0086** | **≤0.01** | 0.75 | **0.012** | **≤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, Rb, and Zn 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 | 87±5 | 96±2 | 94±2 |

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

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.

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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reference | Method | n | Age, yearsM(Range) | Samplepreparation | 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 |
| 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 (Mmin - Mmax),  | 601 – 5772  |
| Ratio Mmax/Mmin | 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.

**Discussion**

As was shown before [21,25,26,47,52] good agreement of the Br, Cu, Fe, I, Rb, Sr, and Zn contents in CRM IAEA H-4 samples analyzed by EDXRF with the certified data of this CRM indicates acceptable accuracy of the results obtained in the study of NT, TBN, and TMN groups of tissue samples presented in Tables 1-6.

From Table 2, it is observed that in TMN tissue the mass fractions of I and Zn are significantly lower while the mass fraction of Rb is higher than in NT and TBN tissue. 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% = 87±5%;

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 [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 87±5% cases (sensitivity).

From Table 2 and Figure 1 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 87±5% cases (sensitivity).

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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reference | Method | n | Age, yearsM(Range) | Samplepreparation | 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 |
| Aeschimann 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 (Mmin - Mmax),  | 77 – 2648  |
| Ratio Mmax/Mmin | 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

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 87, 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].

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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reference | Method | n | Age, yearsM(Range) | Samplepreparation | I, mg/kg dry tissue |
| M±SD | Range |
| Nishida et al 1990 [73] | NAA | 8 | 21-67 | Washed | ≤23±10 | <DL-67 |
| Aeschimann 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 |
| Median of means | 71.8  |
|  Range of means (Mmin - Mmax),  | 23 – 400  |
| Ratio Mmax/Mmin | 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

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].

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 the portable device was used for EDXRF analysis, with its 241Am source for the excitation of X-ray fluorescence in the needle biopsy sample, was developed by ourselves. More powerful devices for EDXRF analysis with X-ray tubes, including “the total reflection” version (TRXRF) of the method, allow reliable determinations of I and many other TEs 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 TEs 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.

**Conclusion**

In this work, TEs analysis was carried out in the tissue samples of NT and thyroid with TBN and TMN using EDXRF. It was shown that EDXRF is an adequate analytical tool for the non-destructive determination of Br, Cu, Fe, I, Rb, Sr, and Zn content 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 Zn were lower while the mean mass fraction of Rb was higher than in NT and TBN tissue. It was demonstrated that I content is 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 study of the I level in a needle biopsy of TNs, obtained by using EDXRF, 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**

No conflict of interest associated with this work.

**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, Dobrean 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 Bedentung der Mengen- und Spurenelemente. 18. Arbeitstangung. 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. Stojsavljević 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.
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](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5385584/) 2017;7:115–119.
14. [Beyersmann D](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Beyersmann%20D%22%5BAuthor%5D), [Hartwig A](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hartwig%20A%22%5BAuthor%5D). Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. [Arch Toxicol](http://www.ncbi.nlm.nih.gov/pubmed/18496671##) 2008;82(8):493-512.
15. [Martinez-Zamudio R](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Martinez-Zamudio%20R%22%5BAuthor%5D), [Ha HC](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Ha%20HC%22%5BAuthor%5D). Environmental epigenetics in metal exposure. [Epigenetics](http://www.ncbi.nlm.nih.gov/pubmed/21610324##) 2011;6(7):820-827.
16. Zaĭchik 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. Zaĭchik 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](https://pubmed.ncbi.nlm.nih.gov/?term=Tsyb+AF&cauthor_id=7741233) AF, [Vtyurin](https://pubmed.ncbi.nlm.nih.gov/?term=Vtyurin+BM&cauthor_id=7741233) 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 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](http://sable.secureserver.net/c/150189?id=5021.90.1.2a76f8b67077921d0bfacc1674a38ea8)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 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 S., Zaichick V. The Br, Fe, Rb, Sr, and Zn contents and interrelation in intact and morphologic normal prostate tissue of adult men investigated by energy-dispersive X-ray fluorescent analysis. X-Ray Spectrom 2011;40(6):464-469.
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.](http://www.scopus.com.scopeesprx.elsevier.com/authid/detail.url?origin=resultslist&authorId=7004288211&zone=), [Zhuk, I.V.](http://www.scopus.com.scopeesprx.elsevier.com/authid/detail.url?origin=resultslist&authorId=7005489709&zone=),[Lomonosova, E.M.](http://www.scopus.com.scopeesprx.elsevier.com/authid/detail.url?origin=resultslist&authorId=6701688172&zone=), [Kanash, N.V.](http://www.scopus.com.scopeesprx.elsevier.com/authid/detail.url?origin=resultslist&authorId=6508072993&zone=), 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](http://www.sciencedirect.com/science/journal/0168583X). 2002;[196(3-4](http://www.sciencedirect.com/science?_ob=PublicationURL&_tockey=%23TOC%235315%232002%23998039996%23354804%23FLA%23&_cdi=5315&_pubType=J&view=c&_auth=y&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=1ea755871e5d8a9eae2188cb0817f7a9)):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](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Hansson%20M%22%5BAuthor%5D), [Grunditz T](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Grunditz%20T%22%5BAuthor%5D), [Isaksson M](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Isaksson%20M%22%5BAuthor%5D), [Jansson S](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Jansson%20S%22%5BAuthor%5D), [Lausmaa J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Lausmaa%20J%22%5BAuthor%5D), [Mölne J](http://www.ncbi.nlm.nih.gov/pubmed?term=%22M%C3%B6lne%20J%22%5BAuthor%5D), [Berg G](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Berg%20G%22%5BAuthor%5D). 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](http://www.ncbi.nlm.nih.gov/pubmed/?term=B%C5%82azewicz%20A%5BAuthor%5D&cauthor=true&cauthor_uid=19944657), [Orlicz-Szczesna G](http://www.ncbi.nlm.nih.gov/pubmed/?term=Orlicz-Szczesna%20G%5BAuthor%5D&cauthor=true&cauthor_uid=19944657), 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 Dis2018b;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 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 innormal 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](https://pubmed.ncbi.nlm.nih.gov/?term=Negretti+de+Br%C3%A4tter+VE&cauthor_id=9857330) VE, [Azzolina](https://pubmed.ncbi.nlm.nih.gov/?term=Azzolina+L&cauthor_id=9857330) 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 inNormal 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 NodulesJ 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 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](http://www.springerlink.com/content/0163-4984/) [2002;90(1-3](http://www.springerlink.com/content/0163-4984/90/1-3/)):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.