

## Diagnosis of thyroid malignancy using trace elements of nodular tissue determined by neutron activation analysis

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### Abstract

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 to evaluate possibilities of using differences in trace elements (TEs) contents in nodular tissue for diagnosis of thyroid malignancy. Contents of TEs such as silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), iodine (I), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), 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 non-destructive instrumental neutron activation analysis. It was observed that in TMN tissue the mean mass fraction of I was lower while the mean mass fraction of Rb was 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. 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

### 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 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”

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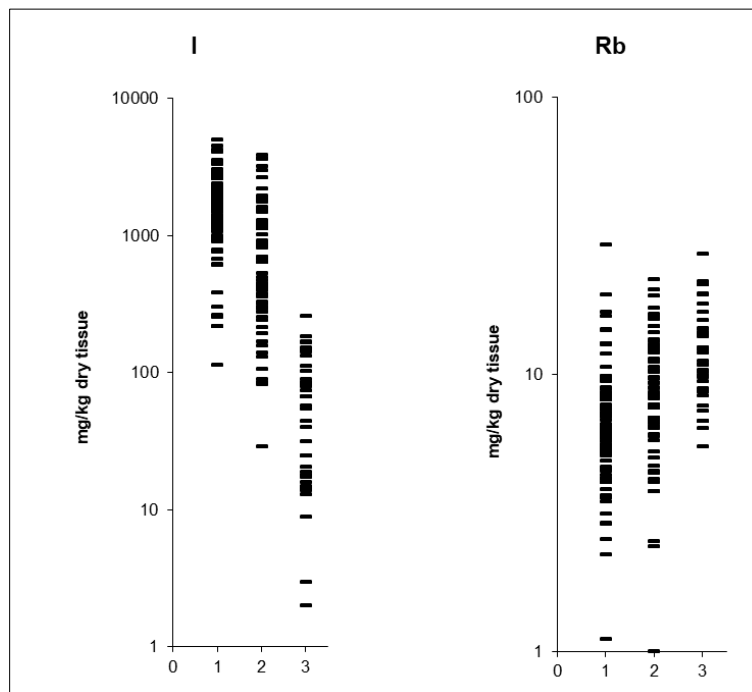


**Table 2** Ratio of means and the difference between mean values Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in normal thyroid (NT) and in thyroid benign (TBN) and malignant (TMN) nodules

| El | TBN and NT   |                 |                 | TMN and NT   |                 |                 | TMN and TBN   |                 |                 |
|----|--------------|-----------------|-----------------|--------------|-----------------|-----------------|---------------|-----------------|-----------------|
|    | 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         | 0.0001          | 0.01            | 12.8         | 0.0001          | 0.01            | 0.854         | 0.515           | >0.05           |
| Co | 1,54         | 0.0001          | 0.01            | 1.38         | 0.0001          | 0.01            | 0.894         | 0.370           | >0.05           |
| Cr | 1,79         | 0.001           | 0.01            | 1.55         | 0.073           | >0.05           | 0.864         | 0.511           | >0.05           |
| Fe | 1,48         | 0.01            | 0.01            | 1.10         | 0.443           | >0.05           | 0.747         | 0.094           | >0.05           |
| Hg | 21,9         | <0.00001        | 0.01            | 19.8         | <0.00001        | 0.01            | 0.903         | 0.567           | >0.05           |
| I  | 0.54         | <0.0001         | 0.01            | 0.039        | <0.0001         | 0.01            | 0.072         | <0.0001         | 0.01            |
| Rb | 1,30         | 0.001           | 0.01            | 1.74         | <0.00001        | 0.01            | 1.340         | 0.00098         | 0.01            |
| Sb | 1,23         | 0.143           | >0.05           | 1.12         | 0.423           | >0.05           | 0.905         | 0.572           | >0.05           |
| Sc | 3,13         | 0.00            | 0.01            | 1.67         | 0.223           | >0.05           | 0.535         | 0.105           | >0.05           |
| Se | 1,19         | 0.174           | >0.05           | 0.88         | 0.235           | >0.05           | 0.742         | 0.039           | 0.01            |
| Zn | 1,21         | 0.008           | 0.01            | 0.97         | 0.839           | >0.05           | 0.806         | 0.111           | >0.05           |

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

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



**Figure 1** Individual data sets for I and Rb mass fractions in samples of normal thyroid (1), thyroid benign nodules (2) and thyroid malignant nodules (3)

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 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

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 [19, 27-30, 52] good agreement of the Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn contents in CRM IAEA H-4 and and CRM IAEA HH-1 samples analyzed by instrumental neutron activation analysis 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 is 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% = 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 [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).

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

| Reference  | Method                        | n   | Age, years<br>M(Range) | Sample<br>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   |
| Blázquez et al. 2011 [63]                              | IC                            | 50  | M=25                   | Fixed                 | 601±192             | 624-4020   |
|  |                               |     |                        | Frozen                | 623±187             | 840 -4000  |
| Zaichick et al. 2017 [27]                              | NAA                           | 72  | 2-80                   | Intact                | 1786±940            | 220-4205   |
| Zaichick et al. 2017 [28]                              | NAA                           | 33  | 3.5-87                 | Intact                | 1956±1199           | 114-5061   |
| Zaichick et al. 2018 [31]                              | EDXRF,NAA                     | 72  | 2-80                   | Intact                | 1786±940            | 220-4205   |
| Zaichick et al. 2018 [32]                              | EDXRF,NAA                     | 33  | 3.5-87                 | Intact                | 1956±1199           | 114-5061   |
| Zaichick et al. 2018 [33]                              | NAA,ICPAES                    | 33  | 3.5-87                 | Intact                | 1956±1199           | 114-5061   |
| Zaichick et al. 2018 [34]                              | NAA,ICPAES                    | 72  | 2-80                   | Intact                | 1786±940            | 220-4205   |
| Zaichick et al. 2018 [37]                              | NAA                           | 105 | 2-80                   | Intact                | 1841±1027           | 114-5061   |
| Zaichick et al. 2018 [64]                              | NAA                           | 105 | 44±21                  | Intact                | 1841±1027           | 114-5061   |
| Zaichick et al. 2018 [65]                              | NAA                           | 105 | 2-80                   | Intact                | 1841±1027           | 114-5061   |
| Zaichick et al. 2018 [66]                              | NAA                           | 105 | 44±21                  | Intact                | 1841±1027           | 114-5061   |
| Zaichick, 2021 [67]                                    | NAA                           | 105 | 2-87                   | Intact                | 1841±1027           | 114-5061   |
| Zaichick, 2021 [68]                                    | NAA                           | 105 | 44±21                  | Intact                | 1841±1027           | 114-5061   |
| Zaichick, 2021 [69]                                    | NAA                           | 105 | 2-87                   | Intact                | 1841±1027           | 114-5061   |
| Zaichick, 2021 [70]                                    | NAA                           | 105 | 44±21                  | Intact                | 1841±1027           | 114-5061   |
| Zaichick, 2021 [71]                                    | NAA,ICPAES                    | 105 | 2-87                   | Intact                | 1841±1027           | 114-5061   |
| Zaichick, 2021 [72]                                    | NAA,ICPAES                    | 105 | 44±21                  | Intact                | 1841±1027           | 114-5061   |
| Median of means  | 1841 mg/kg dry tissue         |     |                        |                       |                     |            |
| Range of means (M <sub>min</sub> - M <sub>max</sub> ), | (601 - 5772) mg/kg dry tissue |     |                        |                       |                     |            |
| Ratio M <sub>max</sub> /M <sub>min</sub>               | 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<br>M(Range) | Sample<br>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 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

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 [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.

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

| Reference                                   | Method                      | n  | Age, years<br>M(Range) | Sample<br>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. 2018 [64]                   | NAA                         | 41 | 16-75                  | Intact                | 71.8±62             | 2-261  |
| Zaichick et al. 2018 [65]                   | EDXRF,NAA                   | 41 | 46±15                  | Intact                | 71.8±62             | 2-261  |
| Zaichick et al. 2018 [66]                   | NAA,ICPAES                  | 41 | 16-75                  | Intact                | 71.8±62             | 2-261  |
| Zaichick, 2022 [81]                         | EDXRF                       | 41 | 16-75                  | Intact                | 71.6±72.5           | 2-341  |
| Zaichick, 2022 [82]                         | NAA                         | 41 | 16-75                  | Intact                | 71.8±62             | 2-261  |
| Zaichick, 2022 [83]                         | NAA                         | 41 | 16-75                  | Intact                | 71.8±62             | 2-261  |
| Zaichick, 2022 [84]                         | EDXRF,NAA                   | 41 | 16-75                  | Intact                | 71.8±62             | 2-261  |
| Zaichick, 2022 [85]                         | 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

## 5. Conclusion

In this work, TES analysis was carried out in the tissue samples of NT and thyroid with TBN and TMN using two methods of neutron activation analysis - INAA-SLR and INAA-LLR. It was shown that neutron activation analysis is an adequate



analytical tool for the non-destructive determination of Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn content 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 fraction of Rb was 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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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The author declares that he has no competing interests.

### *Statement of ethical approval*

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre (MRRC), Obninsk. 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.

### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

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