Content of Fifty Trace Elements in Thyroid Benign Nodules and Thyroid Tissue adjacent to Nodules investigated using Neutron Activation Analysis and Inductively Coupled Plasma Mass Spectrometry

Vladimir Zaichick

Research Article

ABSTRACT
Thyroid benign nodules (TBNs) are the most common diseases of this endocrine gland and are common worldwide. The etiology and pathogenesis of TBNs must be considered as multifactorial. The present study was performed to clarify the role of some trace elements (TEs) in the etiology of these thyroid disorders. Thyroid tissue levels of fifty TEs were prospectively evaluated in nodular tissue and tissue adjacent to nodules of 79 patients with TBNs. Measurements were performed using a combination of non-destructive instrumental neutron activation analysis and destructive method such as inductively coupled plasma mass spectrometry. Results of the study were additionally compared with previously obtained data for the same TEs in “normal” thyroid tissue. This study provides evidence on many TEs level alteration in nodular and adjacent to nodule tissue and shows the necessity to continue TEs research of TBNs.

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Received: January 01, 2022; Accepted: January 07, 2022; Published: January 11, 2022

Keywords: Trace Elements, Thyroid, Thyroid Benign Nodules, Neutron Activation Analysis, Inductively Coupled Plasma Mass Spectrometry

Introduction
Thyroid benign nodules (TBNs) are universally encountered and frequently detected by palpation during a physical examination, or incidentally, during clinical imaging procedures. TBNs include non-neoplastic lesions, for example, colloid goiter and thyroiditis, as well as neoplastic lesions such as thyroid adenomas [1-3]. For over 20th century, there was the dominant opinion that TBNs is the simple consequence of iodine deficiency. However, it was found that TBNs is a frequent disease even in those countries and regions where the population is never exposed to iodine shortage [4]. Moreover, it was shown that iodine excess has severe consequences on human health and associated with the presence of TBNs [5-8]. It was also demonstrated that besides the iodine deficiency and excess many other dietary, environmental, and occupational factors are associated with the TBNs incidence [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 TBNs [12]. Besides iodine, many other TEs have also essential physiological functions. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of TEs depend on tissue-specific need or tolerance, respectively. 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, and thyroid adenoma in comparison with normal thyroid was demonstrated [42-49].

To date, the etiology and pathogenesis of TBNs must be considered as multifactorial. The present study was performed to find out differences in TEs contents between the group of nodular tissues and tissue adjacent to nodules, as well as to clarify the role of some TEs in the etiology of TBNs. Having this in mind, the aim of this exploratory study was to examine differences in the content of silver (Ag), aluminum (Al), arsenic (As), gold (Au), boron (B), beryllium (Be), bismuth (Bi), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), cesium (Cs), dysprosium (Dy), iron (Fe), erbium (Er), europium (Eu), gallium (Ga), gadolinium (Gd), mercury (Hg), holmium (Ho), iridium (Ir), lanthanum (La), lithium (Li), lutecium (Lu), manganese (Mn), molybdenum (Mo), niobium (Nb), neodymium (Nd), nickel (Ni), lead (Pb), palladium (Pd), praseodymium (Pr), platinum (Pt), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), samarium (Sm), tin...
(Sn), terbium (Tb), tellurium (Te), thorium (Th), titanium (Ti), thallium (Tl), thulium (Tm), uranium (U), yttrium (Y), ytterbium (Yb), zinc (Zn), and zirconium (Zr) in nodular and adjacent to nodules tissues of thyroid with TBNs, using a combination of non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) and destructive method such as inductively coupled plasma mass spectrometry (ICP-MS), and to compare the levels of these TEs in two groups (nodular and adjacent to nodules tissues) of the cohort of TBNs samples. Moreover, for understanding a possible role of TEs in etiology and pathogenesis of TBNs results of the study were compared with previously obtained data for the same TEs in “normal” thyroid tissue [42-49].

Material and Methods

All 79 patients suffered from TBNs (46 patients with colloid goiter, mean age M±SD was 48±12 years, range 30-64; 19 patients with thyroid adenoma, mean age M±SD was 41±11 years, range 22-55; and 14 patients with thyroiditis, mean age M±SD was 39±9 years, range 34-50) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre (MRRC), Obninsk. The group of patients with thyroiditis included 8 persons with Hashimoto’s thyroiditis and 6 persons with Riedel’s Struma. 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. For all patients the diagnosis has been confirmed by clinical and morphological/histological results obtained during studies of biopsy and resected materials. “Normal” thyroid for the control group samples were removed at necropsy from 105 deceased (mean age 44±21 years, range 2-87), who had died suddenly. The majority of deaths were due to trauma. 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 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. Titanium tools were used for biopsy, getting tissue samples from resected materials, and sample preparation to prevent contamination by many alloy metals from stainless steel [50]. All tissue samples obtained from nodular tissue and visually “normal” tissue adjacent to nodules were divided into two portions. 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 [51].

The pounded samples weighing about 10 mg (for biopsy) and 100 mg (for resected materials) were used for TEs measurement by INAA-LLR. The content of 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, Pr, Pt, Rh, Sb, Se, Sm, Sn, Tb, Te, Th, Ti, Tm, U, Y, Yb, Zn, and Zr mass fractions measured by ICP-MS were used to determine the Ag, Al, As, Au, B, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Pt, Rh, Sb, Se, Sm, Sn, Tb, Te, Th, Ti, 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 [29,30,35], prostate [52-57], and scalp hair [58].

To determine contents of the TEs by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [59]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. 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.

A dedicated computer program for INAA-LLR mode optimization was used [60]. All thyroid samples 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 INAA-LLR and ICP-MS methods. Using Microsoft Office Excel software, a summary of the statistics, including, arithmetic mean, standard deviation of mean, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for TEs contents in nodular and adjacent tissue of thyroid with TBNs. Data for TEs content in “normal” thyroid were taken from our previous publications [42-49]. The difference in the results between three groups of samples (“normal”, “nodular”, and “adjacent”) was evaluated by the parametric Student’s t-test and non-parametric Wilcoxon-Mann-Whitney U-test.

Results

Table 1 presents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of 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, Pr, Pt, Rh, Sb, Se, Sm, Sn, Tb, Te, Th, Ti, Tm, U, Y, Yb, Zn, and Zr mass fraction in nodular and adjacent to nodules tissue of thyroid with TBN (“nodular” and “adjacent”) group of thyroid tissue samples.

The ratios of means and the comparison of mean values of Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nd, Ni, Pb, Pr, Rh, Sb, Se, Sc, Sm, Sn, Ti, U, Y, and Zn mass fractions in pairs of sample groups such as “normal” and “nodular”, “normal” and “adjacent”, and also “adjacent” and “nodular” are presented in Table 2, 3, and 4, respectively.
Table 1: Some statistical parameters of 50 trace element mass fraction (mg/kg, dry mass basis) in nodular and adjacent tissue of thyroid benign nodules (TBN)

<table>
<thead>
<tr>
<th>Element</th>
<th>Nodular tissue</th>
<th>Adjacent tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Ag</td>
<td>0.192</td>
<td>0.199</td>
</tr>
<tr>
<td>Al</td>
<td>27.3</td>
<td>23.6</td>
</tr>
<tr>
<td>As</td>
<td>&lt;0.004</td>
<td>-</td>
</tr>
<tr>
<td>Au</td>
<td>0.0166</td>
<td>0.0194</td>
</tr>
<tr>
<td>B</td>
<td>4.65</td>
<td>15.0</td>
</tr>
<tr>
<td>Be</td>
<td>0.00090</td>
<td>0.00113</td>
</tr>
<tr>
<td>Bi</td>
<td>0.0706</td>
<td>0.0845</td>
</tr>
<tr>
<td>Cd</td>
<td>1.55</td>
<td>1.68</td>
</tr>
<tr>
<td>Ce</td>
<td>0.0181</td>
<td>0.0176</td>
</tr>
<tr>
<td>Co</td>
<td>0.0576</td>
<td>0.0324</td>
</tr>
<tr>
<td>Cr</td>
<td>1.17</td>
<td>1.19</td>
</tr>
<tr>
<td>Cs</td>
<td>0.0320</td>
<td>0.0471</td>
</tr>
<tr>
<td>Dy</td>
<td>&lt;0.005</td>
<td>-</td>
</tr>
<tr>
<td>Er</td>
<td>0.00303</td>
<td>0.00328</td>
</tr>
<tr>
<td>Eu</td>
<td>&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>Fe</td>
<td>430</td>
<td>566</td>
</tr>
<tr>
<td>Ga</td>
<td>0.0211</td>
<td>0.0081</td>
</tr>
<tr>
<td>Gd</td>
<td>&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>Hg</td>
<td>1.15</td>
<td>1.04</td>
</tr>
<tr>
<td>Ho</td>
<td>&lt;0.0002</td>
<td>-</td>
</tr>
<tr>
<td>Ir</td>
<td>&lt;0.0003</td>
<td>-</td>
</tr>
<tr>
<td>La</td>
<td>0.00939</td>
<td>0.00882</td>
</tr>
<tr>
<td>Li</td>
<td>0.0295</td>
<td>0.0151</td>
</tr>
<tr>
<td>Lu</td>
<td>&lt;0.0002</td>
<td>-</td>
</tr>
<tr>
<td>Mn</td>
<td>1.81</td>
<td>1.41</td>
</tr>
<tr>
<td>Mo</td>
<td>0.193</td>
<td>0.121</td>
</tr>
<tr>
<td>Nb</td>
<td>&lt;0.013</td>
<td>-</td>
</tr>
<tr>
<td>Nd</td>
<td>0.0134</td>
<td>0.0075</td>
</tr>
<tr>
<td>Ni</td>
<td>2.89</td>
<td>2.52</td>
</tr>
<tr>
<td>Pb</td>
<td>1.31</td>
<td>2.27</td>
</tr>
<tr>
<td>Pd</td>
<td>&lt;0.012</td>
<td>-</td>
</tr>
<tr>
<td>Pr</td>
<td>0.00389</td>
<td>0.00335</td>
</tr>
<tr>
<td>Pt</td>
<td>&lt;0.0002</td>
<td>-</td>
</tr>
<tr>
<td>Rb</td>
<td>9.50</td>
<td>4.23</td>
</tr>
<tr>
<td>Sb</td>
<td>0.121</td>
<td>0.108</td>
</tr>
<tr>
<td>Sc</td>
<td>0.0239</td>
<td>0.0383</td>
</tr>
<tr>
<td>Se</td>
<td>3.20</td>
<td>2.92</td>
</tr>
<tr>
<td>Sm</td>
<td>0.00171</td>
<td>0.00181</td>
</tr>
<tr>
<td>Sn</td>
<td>0.0516</td>
<td>0.0399</td>
</tr>
<tr>
<td>Tb</td>
<td>&lt;0.0001</td>
<td>-</td>
</tr>
<tr>
<td>Tc</td>
<td>&lt;0.007</td>
<td>-</td>
</tr>
<tr>
<td>Th</td>
<td>0.0104</td>
<td>0.0155</td>
</tr>
<tr>
<td>Ti</td>
<td>&lt;0.4</td>
<td>-</td>
</tr>
<tr>
<td>Tl</td>
<td>0.00190</td>
<td>0.00109</td>
</tr>
<tr>
<td>Tm</td>
<td>&lt;0.0003</td>
<td>-</td>
</tr>
<tr>
<td>U</td>
<td>0.00116</td>
<td>0.00059</td>
</tr>
</tbody>
</table>

Citation: Vladimir Zaichick (2022) Content of Fifty Trace Elements in Thyroid Benign Nodules and Thyroid Tissue adjacent to Nodules investigated using Neutron Activation Analysis and Inductively Coupled Plasma Mass Spectrometry. Journal of Biotechnology & Bioinformatics Research. SRC/JBBR-145. DOI: doi.org/10.47363/JBBR/2022(4)144
Table 2: Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in normal thyroid (NT) and thyroid benign nodules (TBN) (nodular tissue)

<table>
<thead>
<tr>
<th>Element</th>
<th>NT</th>
<th>TBN nodular</th>
<th>Student's t-test, ( p \leq )</th>
<th>U-test, ( P )</th>
<th>Ratio TBN nodular/NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>0.0133±0.0013</td>
<td>0.192±0.028</td>
<td>0.000001</td>
<td>≤0.01</td>
<td>14.4</td>
</tr>
<tr>
<td>Al</td>
<td>10.5±1.8</td>
<td>27.3±4.2</td>
<td>0.00059</td>
<td>≤0.01</td>
<td>2.60</td>
</tr>
<tr>
<td>B</td>
<td>0.476±0.058</td>
<td>4.65±2.7</td>
<td>0.133</td>
<td>&gt;0.05</td>
<td>9.77</td>
</tr>
<tr>
<td>Be</td>
<td>0.00052±0.00008</td>
<td>0.00090±0.00021</td>
<td>0.093</td>
<td>&gt;0.05</td>
<td>1.73</td>
</tr>
<tr>
<td>Bi</td>
<td>0.0072±0.0022</td>
<td>0.0706±0.0160</td>
<td>0.00050</td>
<td>≤0.01</td>
<td>9.81</td>
</tr>
<tr>
<td>Cd</td>
<td>2.08±0.27</td>
<td>1.55±0.30</td>
<td>0.192</td>
<td>&gt;0.05</td>
<td>0.75</td>
</tr>
<tr>
<td>Ce</td>
<td>0.0080±0.0011</td>
<td>0.0181±0.0030</td>
<td>0.0064</td>
<td>≤0.01</td>
<td>2.26</td>
</tr>
<tr>
<td>Co</td>
<td>0.0390±0.0031</td>
<td>0.0576±0.0045</td>
<td>0.00093</td>
<td>≤0.01</td>
<td>1.48</td>
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<tr>
<td>Cr</td>
<td>0.495±0.031</td>
<td>1.17±0.17</td>
<td>0.00023</td>
<td>≤0.01</td>
<td>2.36</td>
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<tr>
<td>Cs</td>
<td>0.0245±0.0022</td>
<td>0.0320±0.0090</td>
<td>0.423</td>
<td>&gt;0.05</td>
<td>1.31</td>
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<tr>
<td>Er</td>
<td>0.000377±0.000050</td>
<td>0.00303±0.00100</td>
<td>0.00098</td>
<td>≤0.01</td>
<td>8.04</td>
</tr>
<tr>
<td>Fe</td>
<td>222.8±9.6</td>
<td>430±67</td>
<td>0.0031</td>
<td>≤0.01</td>
<td>1.93</td>
</tr>
<tr>
<td>Ga</td>
<td>0.0316±0.0021</td>
<td>0.0211±0.0020</td>
<td>0.00038</td>
<td>≤0.01</td>
<td>0.67</td>
</tr>
<tr>
<td>Hg</td>
<td>0.0543±0.0043</td>
<td>1.15±0.14</td>
<td>0.000001</td>
<td>≤0.01</td>
<td>21.2</td>
</tr>
<tr>
<td>La</td>
<td>0.00475±0.00062</td>
<td>0.00939±0.00200</td>
<td>0.017</td>
<td>≤0.01</td>
<td>1.98</td>
</tr>
<tr>
<td>Li</td>
<td>0.0208±0.0022</td>
<td>0.0295±0.0030</td>
<td>0.018</td>
<td>≤0.01</td>
<td>1.42</td>
</tr>
<tr>
<td>Mn</td>
<td>1.28±0.07</td>
<td>1.81±0.21</td>
<td>0.022</td>
<td>≤0.01</td>
<td>1.41</td>
</tr>
<tr>
<td>Mo</td>
<td>0.0836±0.0062</td>
<td>0.193±0.021</td>
<td>0.000017</td>
<td>≤0.01</td>
<td>2.31</td>
</tr>
<tr>
<td>Nd</td>
<td>0.0041±0.0004</td>
<td>0.0134±0.0020</td>
<td>0.000020</td>
<td>≤0.01</td>
<td>3.27</td>
</tr>
<tr>
<td>Ni</td>
<td>0.449±0.046</td>
<td>2.89±0.47</td>
<td>0.000016</td>
<td>≤0.01</td>
<td>6.44</td>
</tr>
<tr>
<td>Pb</td>
<td>0.233±0.033</td>
<td>1.31±0.41</td>
<td>0.013</td>
<td>≤0.01</td>
<td>5.62</td>
</tr>
<tr>
<td>Pr</td>
<td>0.00107±0.00011</td>
<td>0.00389±0.00100</td>
<td>0.00019</td>
<td>≤0.01</td>
<td>3.64</td>
</tr>
<tr>
<td>Rb</td>
<td>7.54±0.39</td>
<td>9.50±0.50</td>
<td>0.0025</td>
<td>≤0.01</td>
<td>1.26</td>
</tr>
<tr>
<td>Sb</td>
<td>0.0947±0.0075</td>
<td>0.121±0.015</td>
<td>0.122</td>
<td>&gt;0.05</td>
<td>1.28</td>
</tr>
<tr>
<td>Sc</td>
<td>0.0268±0.0060</td>
<td>0.0239±0.0060</td>
<td>0.717</td>
<td>&gt;0.05</td>
<td>0.89</td>
</tr>
<tr>
<td>Se</td>
<td>2.22±0.14</td>
<td>3.20±0.39</td>
<td>0.020</td>
<td>≤0.01</td>
<td>1.44</td>
</tr>
<tr>
<td>Sm</td>
<td>0.000507±0.000064</td>
<td>0.00171±0.00032</td>
<td>0.000492</td>
<td>≤0.01</td>
<td>3.37</td>
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<tr>
<td>Sn</td>
<td>0.0777±0.0091</td>
<td>0.0516±0.0070</td>
<td>0.027</td>
<td>≤0.01</td>
<td>0.66</td>
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<tr>
<td>Tl</td>
<td>0.000932±0.000068</td>
<td>0.00190±0.00020</td>
<td>0.000065</td>
<td>≤0.01</td>
<td>2.04</td>
</tr>
<tr>
<td>U</td>
<td>0.000443±0.000059</td>
<td>0.00116±0.00018</td>
<td>0.0036</td>
<td>≤0.01</td>
<td>2.62</td>
</tr>
<tr>
<td>Y</td>
<td>0.00260±0.00032</td>
<td>0.0110±0.0030</td>
<td>0.0044</td>
<td>≤0.01</td>
<td>4.23</td>
</tr>
<tr>
<td>Zn</td>
<td>94.8±4.2</td>
<td>117.7±5.8</td>
<td>0.0018</td>
<td>≤0.01</td>
<td>1.24</td>
</tr>
</tbody>
</table>

M – arithmetic mean, SEM – standard error of mean, Statistically significant values are in bold.
Table 3: Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in normal thyroid (NT) and thyroid benign nodules (TBN) (adjacent tissue)

<table>
<thead>
<tr>
<th>Element</th>
<th>Thyroid tissue</th>
<th>TBN adjacent</th>
<th>Student’s t-test, ( p \leq )</th>
<th>U-test, ( p )</th>
<th>Ratio TBN adjacent/NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>0.0133±0.0013</td>
<td>0.473±0.130</td>
<td>0.0016</td>
<td>( \leq 0.01 )</td>
<td>35.6</td>
</tr>
<tr>
<td>Al</td>
<td>10.5±1.8</td>
<td>25.7±7.1</td>
<td>0.120</td>
<td>( &gt;0.05 )</td>
<td>2.45</td>
</tr>
<tr>
<td>B</td>
<td>0.476±0.058</td>
<td>1.70±0.35</td>
<td>0.070</td>
<td>( &gt;0.05 )</td>
<td>3.57</td>
</tr>
<tr>
<td>Be</td>
<td>0.00052±0.00008</td>
<td>0.00053±0.00002</td>
<td>0.909</td>
<td>( &gt;0.05 )</td>
<td>1.02</td>
</tr>
<tr>
<td>Bi</td>
<td>0.0072±0.0022</td>
<td>0.478±0.226</td>
<td>0.129</td>
<td>( &gt;0.05 )</td>
<td>66.4</td>
</tr>
<tr>
<td>Cd</td>
<td>2.08±0.27</td>
<td>2.46±0.75</td>
<td>0.653</td>
<td>( &gt;0.05 )</td>
<td>1.18</td>
</tr>
<tr>
<td>Ce</td>
<td>0.0080±0.0011</td>
<td>0.488±0.469</td>
<td>0.382</td>
<td>( &gt;0.05 )</td>
<td>61.0</td>
</tr>
<tr>
<td>Co</td>
<td>0.0390±0.0031</td>
<td>0.0733±0.0170</td>
<td>0.052</td>
<td>( \leq 0.05 )</td>
<td>1.88</td>
</tr>
<tr>
<td>Cr</td>
<td>0.495±0.031</td>
<td>0.614±0.111</td>
<td>0.311</td>
<td>( &gt;0.05 )</td>
<td>1.24</td>
</tr>
<tr>
<td>Cs</td>
<td>0.0245±0.0022</td>
<td>0.0132±0.0030</td>
<td>0.045</td>
<td>( \leq 0.01 )</td>
<td>0.54</td>
</tr>
<tr>
<td>Er</td>
<td>0.000377±0.000050</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>222.8±9.6</td>
<td>217±24</td>
<td>0.836</td>
<td>( &gt;0.05 )</td>
<td>0.97</td>
</tr>
<tr>
<td>Ga</td>
<td>0.0316±0.0021</td>
<td>( \leq 0.024 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hg</td>
<td>0.0543±0.0043</td>
<td>1.39±0.16</td>
<td>0.0000000003</td>
<td>( \leq 0.01 )</td>
<td>25.6</td>
</tr>
<tr>
<td>La</td>
<td>0.00475±0.00062</td>
<td>( \leq 0.088 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Li</td>
<td>0.0208±0.0022</td>
<td>0.0350±0.0110</td>
<td>0.288</td>
<td>( &gt;0.05 )</td>
<td>1.68</td>
</tr>
<tr>
<td>Mn</td>
<td>1.28±1.07</td>
<td>1.78±0.36</td>
<td>0.188</td>
<td>( &gt;0.05 )</td>
<td>1.39</td>
</tr>
<tr>
<td>Mo</td>
<td>0.0836±0.0062</td>
<td>0.127±0.031</td>
<td>0.247</td>
<td>( &gt;0.05 )</td>
<td>1.52</td>
</tr>
<tr>
<td>Nd</td>
<td>0.0041±0.0004</td>
<td>( \leq 0.048 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>0.449±0.046</td>
<td>1.71±0.84</td>
<td>0.268</td>
<td>( &gt;0.05 )</td>
<td>3.81</td>
</tr>
<tr>
<td>Pb</td>
<td>0.233±0.033</td>
<td>0.83±0.35</td>
<td>0.190</td>
<td>( &gt;0.05 )</td>
<td>3.56</td>
</tr>
<tr>
<td>Pr</td>
<td>0.00107±0.00011</td>
<td>( \leq 0.017 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rb</td>
<td>7.5±0.39</td>
<td>10.4±0.7</td>
<td>0.0012</td>
<td>( \leq 0.01 )</td>
<td>1.38</td>
</tr>
<tr>
<td>Sb</td>
<td>0.0947±0.0075</td>
<td>0.131±0.030</td>
<td>0.251</td>
<td>( &gt;0.05 )</td>
<td>1.26</td>
</tr>
<tr>
<td>Sc</td>
<td>0.0268±0.0060</td>
<td>0.0058±0.0020</td>
<td>0.0024</td>
<td>( \leq 0.01 )</td>
<td>0.22</td>
</tr>
<tr>
<td>Se</td>
<td>2.22±0.14</td>
<td>1.93±0.15</td>
<td>0.149</td>
<td>( &gt;0.05 )</td>
<td>0.87</td>
</tr>
<tr>
<td>Sm</td>
<td>0.000507±0.000064</td>
<td>( \leq 0.0011 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sn</td>
<td>0.0777±0.0091</td>
<td>0.135±0.060</td>
<td>0.407</td>
<td>( &gt;0.05 )</td>
<td>1.74</td>
</tr>
<tr>
<td>Ti</td>
<td>0.000932±0.000068</td>
<td>( \leq 0.0036 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>0.000443±0.000059</td>
<td>0.00180±0.00049</td>
<td>0.068</td>
<td>( &gt;0.05 )</td>
<td>4.06</td>
</tr>
<tr>
<td>Y</td>
<td>0.00260±0.00032</td>
<td>0.0088±0.0030</td>
<td>0.145</td>
<td>( &gt;0.05 )</td>
<td>3.38</td>
</tr>
<tr>
<td>Zn</td>
<td>94.8±4.2</td>
<td>105±12</td>
<td>0.409</td>
<td>( &gt;0.05 )</td>
<td>1.11</td>
</tr>
</tbody>
</table>

M – arithmetic mean, SEM – standard error of mean, Statistically significant values are in bold.
Table 4: Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in nodular and adjacent tissue of thyroid benign nodules (TBN)

<table>
<thead>
<tr>
<th>Element</th>
<th>Thyroid tissue</th>
<th>TBN nodular</th>
<th>TBN adjacent</th>
<th>Student’s t-test</th>
<th>U-test</th>
<th>Ratio</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td>0.473±0.130</td>
<td>0.192±0.028</td>
<td></td>
<td>0.044</td>
<td>≤0.01</td>
<td>0.41</td>
</tr>
<tr>
<td>Al</td>
<td>25.7±7.1</td>
<td>27.3±4.2</td>
<td></td>
<td>0.848</td>
<td>&gt;0.05</td>
<td>1.06</td>
</tr>
<tr>
<td>B</td>
<td>1.70±0.35</td>
<td>4.65±2.7</td>
<td></td>
<td>0.287</td>
<td>&gt;0.05</td>
<td>2.74</td>
</tr>
<tr>
<td>Be</td>
<td>0.00053±0.00002</td>
<td>0.00090±0.00021</td>
<td></td>
<td>0.083</td>
<td>&gt;0.05</td>
<td>1.70</td>
</tr>
<tr>
<td>Bi</td>
<td>0.478±0.226</td>
<td>0.0706±0.0160</td>
<td></td>
<td>0.169</td>
<td>&gt;0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>Cd</td>
<td>2.46±0.75</td>
<td>1.55±0.30</td>
<td></td>
<td>0.316</td>
<td>&gt;0.05</td>
<td>0.63</td>
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<tr>
<td>Ce</td>
<td>0.488±0.469</td>
<td>0.0181±0.0030</td>
<td></td>
<td>0.390</td>
<td>&gt;0.05</td>
<td>0.037</td>
</tr>
<tr>
<td>Co</td>
<td>0.073±0.0170</td>
<td>0.0576±0.0045</td>
<td></td>
<td>0.370</td>
<td>&gt;0.05</td>
<td>0.79</td>
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<tr>
<td>Cr</td>
<td>0.614±0.111</td>
<td>1.17±0.17</td>
<td>0.0070</td>
<td>≤0.01</td>
<td>1.91</td>
<td></td>
</tr>
<tr>
<td>Cs</td>
<td>0.0132±0.0030</td>
<td>0.0320±0.0090</td>
<td></td>
<td>0.057</td>
<td>&gt;0.05</td>
<td>2.42</td>
</tr>
<tr>
<td>Er</td>
<td>&lt;0.001</td>
<td>0.00030±0.00100</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>217±24</td>
<td>430±67</td>
<td>0.0037</td>
<td>≤0.01</td>
<td>1.98</td>
<td></td>
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<tr>
<td>Ga</td>
<td>≤0.024</td>
<td>0.0211±0.0020</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Hg</td>
<td>1.39±0.16</td>
<td>1.15±0.14</td>
<td>0.274</td>
<td>&gt;0.05</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>La</td>
<td>≤0.088</td>
<td>0.00939±0.00200</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Li</td>
<td>0.0350±0.0110</td>
<td>0.0295±0.0030</td>
<td></td>
<td>0.656</td>
<td>&gt;0.05</td>
<td>0.84</td>
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<tr>
<td>Mn</td>
<td>1.78±0.36</td>
<td>1.81±0.21</td>
<td>0.946</td>
<td>&gt;0.05</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td>Mo</td>
<td>0.127±0.031</td>
<td>0.193±0.021</td>
<td>0.123</td>
<td>&gt;0.05</td>
<td>1.52</td>
<td></td>
</tr>
<tr>
<td>Nd</td>
<td>≤0.048</td>
<td>0.0134±0.0020</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>1.71±0.84</td>
<td>2.89±0.47</td>
<td>0.292</td>
<td>&gt;0.05</td>
<td>1.69</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.83±0.35</td>
<td>1.31±0.41</td>
<td>0.385</td>
<td>&gt;0.05</td>
<td>1.58</td>
<td></td>
</tr>
<tr>
<td>Pr</td>
<td>≤0.017</td>
<td>0.00389±0.00100</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Rb</td>
<td>10.4±0.7</td>
<td>9.50±0.50</td>
<td>0.306</td>
<td>&gt;0.05</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Sb</td>
<td>0.131±0.030</td>
<td>0.121±0.015</td>
<td>0.759</td>
<td>&gt;0.05</td>
<td>0.92</td>
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<tr>
<td>Sc</td>
<td>0.0058±0.0020</td>
<td>0.0239±0.0060</td>
<td></td>
<td>0.0040</td>
<td>≤0.01</td>
<td>4.12</td>
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<tr>
<td>Se</td>
<td>1.93±0.15</td>
<td>3.20±0.39</td>
<td>0.0033</td>
<td>≤0.01</td>
<td>1.66</td>
<td></td>
</tr>
<tr>
<td>Sm</td>
<td>≤0.0011</td>
<td>0.00171±0.00032</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sn</td>
<td>0.135±0.060</td>
<td>0.0516±0.0070</td>
<td></td>
<td>0.256</td>
<td>&gt;0.05</td>
<td>0.38</td>
</tr>
<tr>
<td>Ti</td>
<td>≤0.0036</td>
<td>0.00190±0.00020</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>0.00180±0.00049</td>
<td>0.00116±0.00018</td>
<td></td>
<td>0.290</td>
<td>&gt;0.05</td>
<td>0.64</td>
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<tr>
<td>Y</td>
<td>0.0088±0.0030</td>
<td>0.0110±0.0030</td>
<td></td>
<td>0.594</td>
<td>&gt;0.05</td>
<td>1.25</td>
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<td>Zn</td>
<td>10.5±12</td>
<td>117.7±5.8</td>
<td>0.351</td>
<td>&gt;0.05</td>
<td>1.12</td>
<td></td>
</tr>
</tbody>
</table>

M – arithmetic mean, SEM – standard error of mean, Statistically significant values are in bold.

Discussion
As was shown before [29,30,35,52-58] good agreement of the 50 TE mass fractions in CRM IAEA H-4, INCT-SBF-4, INCT-TL-1, and INCT-MPH-2 samples determined by both INAA-LLR and ICP-MS methods with the certified data of these CRMs indicates acceptable accuracy of the results obtained in the study of thyroid tissue samples presented in Tables 1–4.

The Ag, Al, Bi, Ce, Co, Cr, Er, Fe, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sc, Sm, Ti, U, Y, and Zn contents in “nodular” tissue were higher, while Ga and Sn content were lower in comparison with contents of these TEs in normal gland (Table 2). Significant differences between TEs contents of “normal” thyroid and TEs contents of thyroid tissue adjacent to nodules were found for Ag, Cs, Hg, Rb, and Sc. Mass fractions of Ag, Hg, and Rb in “adjacent” group of samples were approximately 36, 26, and 1.4 times, respectively, higher, while Sc content was almost 5 times lower than in “normal” thyroid (Table 3). In a general sense Al, B, Be, Bi, Cd, Ce, Co, Cs, Ga, Hg, Li, Mn, Mo, Ni, Pb, Rb, Sb, Sn, U, Y, and Zn contents found in the “nodular” and “adjacent” groups of thyroid tissue samples were very similar (Table 4). However, levels of Cr, Fe, Sc, and Se were lower, while content of Ag in “adjacent” group of samples was higher than in nodular tissue (Table 4).

Characteristically, elevated or reduced levels of TEs observed in thyroid nodules are discussed in terms of their potential role in the initiation and promotion of these thyroid lesions. In other words, using the low or high levels of the TEs in affected thyroid tissues researchers try to determine the role of the deficiency or excess of TEs in the development of thyroid diseases.
excess of each TEs in the etiology and pathogenesis of thyroid diseases. In our opinion, abnormal levels of many TEs in TBNs could be and cause, and also effect of thyroid tissue transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in TEs level in pathologically altered tissue is the reason for alterations or vice versa. According to our opinion, investigation of TEs contents in thyroid tissue adjacent to nodules and comparison obtained results with TEs levels typical of “normal” thyroid gland may give additional useful information on the topic because this data show conditions of tissue in which TBNs were originated and developed. For example, results of this study demonstrates that contents Ag, Hg, and Rb in thyroid tissue in which TBNs were originated and developed were significantly higher the levels which are “normal” for thyroid gland.

Silver
Ag is a TE with no recognized trace metal value in the human body [61]. Food is the major intake source of Ag and this metal is authorised as a food additive (E174) in the EU [62]. Another source of Ag is contact with skin and mucosal surfaces because Ag is widely used in different applications (e.g., jewelry, wound dressings, or eye drops) [63]. Ag in metal form and inorganic Ag compounds ionize in the presence of water, body fluids or tissue exudates. The silver ion Ag+ is biologically active and readily interacts with proteins, amino acid residues, free anions and receptors on mammalian and eukaryotic cell membranes [64]. Besides such the adverse effects of chronic exposure to Ag as a permanent bluish-gray discoloration of the skin (argyria) or eyes (argyrosis), exposure to soluble Ag compounds may produce other toxic effects, including liver and kidney damage, irritation of the eyes, skin, respiratory, and intestinal tract, and changes in blood cells [65]. Experimental studies shown that Ag nanoparticles may affect thyroid hormone metabolism [66]. More detailed knowledge of the Ag toxicity can lead to a better understanding of the impact on human health, including thyroid function.

Mercury
In the general population, potential sources of Hg exposure include the inhalation of this metal vapor in the air, ingestion of contaminated foods and drinking water, and exposure to dental amalgam through dental care [67]. Hg is one of the most dangerous environmental pollutants [68]. The growing use of this metal in diverse areas of industry has resulted in a significant increase of environment contamination and episodes of human intoxication. Many experimental and occupational studies of Hg in different chemical states shown significant alterations in thyroid hormones metabolism and thyroid gland parenchyma [69,70]. Moreover, Hg was classified as certain or probable carcinogen by the International Agency for Research on Cancer [71]. For example, in Hg polluted area thyroid cancer incidence was almost 2 times higher than in adjacent control areas [72].

Rubidium
There is very little information about Rb effects on thyroid function. Rb as a monovalent cation Rb+ is transferred through membrane by the Na+K+-ATPase or H+K+ - ATPase pump membrane transport systems for monovalent cations, which can be stimulated by endocrin system, including thyroid hormones [74]. It was found also that Rb has some function in immune responce [75] and that elevated concentration of Rb could modulate proliferative responses of the cell, as was shown for bone marrow leukocytes [76]. These data partially clarify the possible role of Rb in etiology and pathogenesis of TBNs.

Iron
It is well known that Fe as TEs is involved in many very important functions and biochemical reactions of human body. Fe metabolism is therefore very carefully regulated at both a systemic and cellular level [77,78]. Under the impact of age and multiple environmental factors the Fe metabolism may become dysregulated with attendant accumulation of this metal excess in tissues and organs, including thyroid [25,26,29-35]. Most experimental and epidemiological data support the hypothesis that Fe overload is a risk factor for benign and malignant tumors [79]. This goitrogenic and oncogenic effect could be explained by an overproduction of ROS and free radicals [80]. Thus, on the one hand, the accumulated data suggest that Fe might be responsible for TBNs development. But, on the other hand, the elevated level of Fe was not found in thyroid tissue adjacent to nodules. It is well known that blood is the main pool for Fe in human body and therefore high vascularisation of nodular tissue may be the reason for Fe elevated levels in TBNs [81].

Selenium
The high level of Se content found just in the TBNs cannot be regarded as pure chance. The seleno-protein characterized as Se-dependent glutathione peroxidase (Se-GSH-Px) is involved in protecting cells from peroxidative damage. This enzyme may reduce tissue concentration of free radicals and hydroperoxides. It is particular important for the thyroid gland, because thyroidal functions involve oxidation of iodide, which is incorporated into thyreoglobulin, the precursor of the thyroid hormones. For oxidation of iodide thyroidal cells produce a specific thyroid peroxidase using of physiologically generated hydrogen-peroxide (H2O2) as a cofactor [82]. It follows that the thyroid parenchyma must be continuously exposed to a physiological generation of H2O2 and in normal conditions must be a balance between levels of Se (as Se-GSH-Px) and H2O2. The elevated level of Se was not found in thyroid tissue adjacent to nodules but in nodular tissue. Thus, it might be assumed that the elevated level of Se in TBNs tissue reflects an increase in concentration of free radicals and hydroperoxides during nodular transformation.

This study has several limitations. Firstly, analytical techniques employed in this study measure only fifty TEs mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of TEs investigated in “normal” thyroid and in pathologically altered tissue. Secondly, the sample size of TBNs group was relatively small and prevented investigations of TEs contents in this group using differentials like gender, histological types of TBNs, nodules functional activity, stage of disease, and dietary habits of patients with TBNs. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on many TEs level alteration in nodular and adjacent to nodule tissue and shows the necessity to continue TEs research of TBNs.
Conclusion
In this work, TEs analysis was carried out in the tissue samples of TBNs (nodular and adjacent to nodules) using a combination of non-destructive INAA-LLR and destructive ICP-MS methods. It was shown that this combination is an adequate analytical tool for the determination of fifty TEs content in the tissue samples of human thyroid in norm and pathology, including needle-biopsy specimens. It was observed that Ag, Al, Bi, Ce, Co, Cr, Er, Fe, Hg, La, Li, Mn, Mo, Nd, Ni, Ph, Pr, Rb, Sc, Sm, Tb, U, and Zn contents in “nodular” tissue were higher, while Ga and Sn content were lower in comparison with contents of these TEs in normal gland. Mass fractions of Ag, Hg, and Rb in “adjacent” group of samples were approximately 36, 26, and 1.4 times, respectively, higher, while Sc content was almost 5 times lower than in “normal” thyroid. Contents of Al, B, Be, Bi, Cd, Ce, Co, Cs, Ga, Hg, Li, Mn, Mo, Ni, Pb, Rb, Sb, Sn, U, and Zn contents found in the “nodular” and “adjacent” groups of thyroid tissue samples were very similar. However, levels of Cr, Fe, Sc, and Se were lower, while content of Ag in “adjacent” group of samples was higher than in nodular tissue.

Acknowledgements
The author is extremely grateful to Profs. Vtyurin BM and Medvedev VS, Medical Radiological Research Center, Obninsk, as well as to Dr. Choporov Yu, former Head of the Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples. The author is also grateful to Dr. Karandaschev V, Dr. Nosenko S, and Moskova I, Institute of Microelectronics Technology and High Purity Materials, Chernogolovka, Russia, for their help in ICP-MS analysis.

Funding
There were no any sources of funding that have supported this work.

Conflict of Interest
The author has not declared any conflict of interests.

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