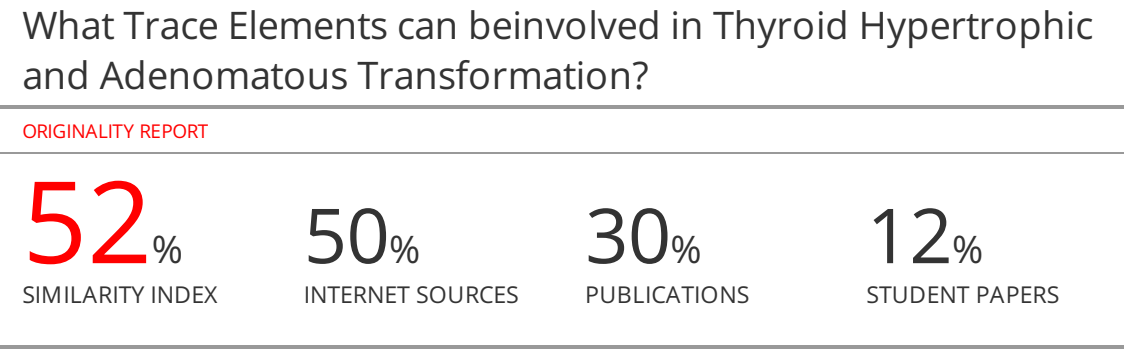
**Reviewer’s Comments**

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**What Trace Elements can be involved in Thyroid Hypertrophic and Adenomatous Transformation?**

**Abstract**

**Background**: Thyroid benign nodules (TBNs) are the most common lesions of this endocrine gland and are prevalent diseases around the world. Among TBNs the colloid goiter (CG) and thyroid adenoma (TA) are very frequent diseases. An evaluation of the variant of TBNs is clinically important for subsequent therapeutic interventions, as well as for more clear understanding the etiology of these disorders. The aim of this exploratory study was to examine differences in the content of fifty trace elements (TE) in tissues of CG and TA. **Method**s: Thyroid tissue levels of TE were prospectively evaluated in 46 patients with CG and 19 patients with TA. Measurements were performed using a combination of non-destructive and destructive methods: instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) and inductively coupled plasma mass spectrometry (ICPMS), respectively. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for TE analysis. **Results**: It was observed that in both CG and TA tissues contents of Ag, Al, Cr, Hg, Mn, Th, and Zn increased, whereas levels of Au, Be, Cs, Pb, Rb, Sb, Sc, Th, Yb, and Zr did not changed in comparison with normal thyroid tissue. It was not found any differences between TE contents of CG and TA. **Conclusions**: From obtained results it was possible to conclude that the common characteristics of CG and TA tissue samples were elevated level of Ag, Al, Cr, Hg, Mn, Th, and Zn in comparison with normal thyroid and, therefore, these TE can be involved in etiology and pathogenesis of such thyroid disorders as CG and TA.

**Keywords:** Thyroid; Thyroid colloid goiter; Thyroid adenoma;Traceelements;Neutron activation analysis; Inductively coupled plasma mass spectrometry

**Introduction**

Thyroid benign nodules (TBNs) are the most common lesions of this endocrine gland that encountered globally and frequently discovered by palpation during a physical examination, or incidentally, during clinical imaging procedures. TBNs include non-neoplastic lesions, for example, colloid goiter (CG) and neoplastic lesion such as thyroid adenoma (TA) [1-3]. An evaluation of the variant of TBNs is clinically important for subsequent therapeutic interventions. For this reason the finding of specific characteristics of CG and TA is the barest necessity for the differential diagnosis of these thyroid disorders.

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 thatiodine excess has severe consequences on human health and associated with the presence of TBNs [5-8]. It was also demonstrated that besides the iodine deficiencyand excess many other dietary, environmental, and occupational factors are associated with the TBNs incidence [9-11]. Among these factorsa disturbance of evolutionary stable input of many trace elements (TE) in human body after industrial revolution plays a significant role in etiology of TBNs[12].

Besides iodine, many other TE have also essential physiological functions [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of TEdepend on tissue-specific need or tolerance, respectively [13].Excessive accumulation or an imbalance of the TEmay disturb the cell functions and may result in cellular 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 TE contents in the normal and pathological thyroid [16-22]. Iodine levelin the normal thyroid was investigated in relation to age, gender and some non-thyroidal diseases [23,24]. After that, variations ofmanyTE content with age in the thyroid of males and femaleswere studied and age- and gender-dependence of some TEwas observed [25-41]. Furthermore, a significant difference between some TEcontents in CGand TA in comparison with normal thyroid was demonstrated [42-44].

To date, the etiology and pathogenesis of CG and TA has to be considered as multifactorial. The present study was performed to find differences in TE contents between CG and TA group of samples, as well as to clarify the role of some TE in the etiology of these thyroid lesions. Having this in mind, our aim was to assess the 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)contents in CG and TAtissue samples using a combination of non-destructive and destructive methods: instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) and inductively coupled plasma mass spectrometry (ICP-MS), respectively. A further aim was to compare the levels of these TE in CG and TA group of samples.

**Material and Methods**

All patients suffered from CG (n=46, mean age M±SD was 48±12 years, range 30-64) and TA(n=19, mean age M±SD was 41±11 years, range 22-55) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre. 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 TE contents. For all patients the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials (46 euthyroid CG, 4 toxic TA and 15 non-toxic TA). Histological conclusion for all thyroidal lesions was the CG (16 macro-follicular,13 micro-follicular, and 17 macro-micro-follicular) and TA (4 macro-follicular, 4 micro-follicular, 11 macro-micro follicular).

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre (MRRC), Obninsk (Reference number 115050610007, year 2017). All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/ornational research committee and with the 1964 Helsinki declaration and its later amendments, or with comparable ethical standards

All tissue samples were divided into two portions using a titanium scalpel [45]. One was used for morphological study while the other was intended for TE analysis. After the samples intended for TE analysis were weighed, they were freeze-dried and homogenized [46].

The pounded samples weighing about 10 mg (for biopsy) and 100 mg (for resected materials) were used for ChE measurement by INAA-LLR. The content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn were determined by INAA-LLR using a vertical channel of the WWR-c research nuclear reactor (Branch of Karpov Institute, Obninsk). After non-destructive INAA-LLR investigation the thyroid samples were used for ICP-MS. The samples were decomposed in autoclaves and aliquots of solutions were used to determine the Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr mass fractions by ICP-MS using an ICP-MS Thermo-Fisher “X-7” Spectrometer (Thermo Electron, USA).Information detailing with the NAA-LLR and ICP-MS methods used and other details of the analysis were presented in our earlier publications concerning TE contents in human thyroid, prostate, and scalp hair [29,30,35,47-53].

To determine contents of the TE by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [54].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-2Mixed 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-SLRmode optimization was used [55].All thyroid samples were prepared in duplicate, and mean values of TE contents were used in final calculation. Mean values of TE contents were used in final calculation for the Ag, Co, Cr, Hg, Rb, Sb, Se, and Zn mass fractions measured by two methods. Using Microsoft Office Excel software, a summary of the statistics, including, arithmetic mean, standard deviation, standard error of mean, and range (minimal - maximal value), was calculated for TE contents in CG and TA tissue samples. The difference in the results between two groups of samples 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, and range) of the Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Fe, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Sc, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zrmass fractionin CG and TA tissue samples.

The ratios of means and the comparison of mean values of Ag, Al, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Er, Fe,Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc,Se, Sm, Sn, Tl, U, Y, Yb, Zn, and Zrmass fractions in CG and TAare presented in Table 2.

Table 3 depicts the results of comparison the contents of Ag, Al, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Er, Fe,Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc,Se, Sm, Sn, Tl, U, Y, Yb, Zn, and Zrin CG and TA sample groups with those in normal thyroid(from data analysis of previous publications [43,44]), as well as comparison the contents of these ChE in CG and TA sample groups.

**Discussion**

As was shown before [29,30,35,47-53] 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 CG and TA samples and presented in Tables 1–3.

In a general sense variations found for Ag, Al, Au, Be, Cr, Cs, Hg, Mn, Pb, Rb, Sb,Sc, Th, Yb, Zn, and Zr contents in CG and TA tissue samples were similar in comparison with normal thyroid tissue (Table 3). In affected tissues contents of Ag, Al, Cr, Hg, Mn, Th, and Zn increased, whereas levels of Au, Be, Cs, Pb, Rb, Sb, Sc, Th, Yb, and Zrdid not changed in both groups of samples (Table 3).There was not found any differences between TE contents of CG and TA, when results for these groups were compared with each other (Tables 2 and 3).

**Table 1**. Some statistical parameters of 50 trace element mass fraction (mg/kg, dry mass basis) in the thyroid colloid goiter and adenoma

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Element | Colloid nodular goiter (n=46) | | | Adenoma (n=19) | | |
| M | SD | Range | M | SD | Range |
| Ag | 0.192 | 0.214 | 0.002-0.842 | 0.181 | 0.180 | 0.0012-0.6790 |
| Al | 27.1 | 24.7 | 6.6-95.1 | 34.2 | 24.1 | 8.7-78.4 |
| As | <0.004 | - | - | <0.004 | - | - |
| Au | 0.0141 | 0.0152 | 0.0030-0.0585 | 0.0287 | 0.0293 | 0.0030-0.0709 |
| B | 5.50 | 17.8 | 0.9-85.2 | 3.38 | 2.74 | 1.00-7.30 |
| Be | 0.00072 | 0.00053 | 0.0002-0.0020 | 0.00181 | 0.00222 | 0.00020-0.00600 |
| Bi | 0.0585 | 0.0560 | 0.0039-0.2140 | 0.112 | 0.157 | 0.0113-0.4220 |
| Cd | 1.26 | 1.30 | 0.126-5.360 | 2.78 | 2.51 | 0.31-6.39 |
| Ce | 0.0186 | 0.0185 | 0.0031-0.0696 | 0.0246 | 0.0174 | 0.0073-0.0459 |
| Co | 0.0576 | 0.0282 | 0.015-0.147 | 0.0660 | 0.0469 | 0.0159-0.1590 |
| Cr | 1.18 | 1.38 | 0.144-7.300 | 1.36 | 0.82 | 0.259-2.79 |
| Cs | 0.0216 | 0.0232 | 0.0076-0.1140 | 0.052 | 0.085 | 0.0111-0.205 |
| Dy | <0.005 | - | - | <0.005 | - | - |
| Er | 0.00299 | 0.00332 | 0.0010-0.0138 | 0.00400 | 0.00390 | 0.0010-0.0090 |
| Eu | <0.001 | - | - | <0.001 | - | - |
| Fe | 449 | 597 | 62-2734 | 571 | 675 | 52.3-2563.0 |
| Ga | 0.0210 | 0.0080 | 0.0100-0.0340 | 0.0223 | 0.0097 | 0.0100-0.0300 |
| Gd | <0.001 | - | - | <0.001 | - | - |
| Hg | 1.18 | 1.01 | 0.10-5.18 | 1.16 | 1.26 | 0.193-5.200 |
| Ho | <0.0002 | - | - | <0.0002 | - | - |
| Ir | <0.0003 | - | - | <0.0003 | - | - |
| La | 0.00990 | 0.00921 | 0.0017-0.0356 | 0.0116 | 0.0105 | 0.0054-0.0237 |
| Li | 0.0281 | 0.0117 | 0.0073-0.0541 | 0.0401 | 0.0236 | 0.0185-0.0680 |
| Lu | <0.0002 | - | - | <0.0002 | - | - |
| Mn | 1.77 | 1.13 | 0.45-5.50 | 1.67 | 1.88 | 0.10-6.12 |
| Mo | 0.183 | 0.121 | 0.049-0.627 | 0.233 | 0.145 | 0.046-0.448 |
| Nb | <0.013 | - | - | <0.013 | - | - |
| Nd | 0.0139 | 0.0087 | 0.0031-0.0331 | 0.0141 | 0.0047 | 0.0096-0.0190 |
| Ni | 2.63 | 2.43 | 0.13-10.40 | 3.95 | 3.39 | 0.48-9.00 |
| Pb | 0.94 | 1.86 | 0.12-8.90 | 1.86 | 3.29 | 0.26-9.30 |
| Pd | <0.012 | - | - | <0.012 | - | - |
| Pr | 0.00396 | 0.00359 | 0.00053-0.01310 | 0.00475 | 0.00345 | 0.0012-0.0093 |
| Pt | <0.0002 | - | - | <0.0002 | - | - |
| Rb | 9.50 | 4.23 | 2.5-22.1 | 8.96 | 3.19 | 3.6-16.4 |
| Sb | 0.127 | 0.113 | 0.00102-0.42500 | 0.140 | 0.117 | 0.0449-0.4660 |
| Sc | 0.0196 | 0.0316 | 0.0002-0.1130 | 0.0286 | 0.0451 | 0.0003-0.1400 |
| Se | 3.54 | 3.31 | 0.86-13.80 | 3.01 | 2.43 | 0.72-10.60 |
| Sm | 0.00169 | 0.00156 | 0.00040-0.00690 | 0.00252 | 0.00263 | 0.0004-0.0080 |
| Sn | 0.0458 | 0.0384 | 0.0143-0.1720 | 0.0756 | 0.0443 | 0.0331-0.1570 |
| Tb | <0.0001 | - | - | <0.0001 | - | - |
| Te | <0.007 | - | - | <0.007 | - | - |
| Th | 0.0074 | 0.0062 | 0.0020-0.0210 | 0.0229 | 0.0293 | 0.0020-0.0783 |
| Ti | <0.4 | - | - | <0.4 | - | - |
| Tl | 0.00174 | 0.00093 | 0.00052-0.00350 | 0.00238 | 0.00164 | 0.0011-0.0054 |
| Tm | <0.0003 | - | - | <0.0003 | - | - |
| U | 0.00145 | 0.00053 | 0.00082-0.00240 | 0.00083 | 0.00035 | 0.00044-0.00110 |
| Y | 0.0113 | 0.0103 | 0.0036-0.0346 | 0.0115 | 0.0140 | 0.0031-0.0361 |
| Yb | 0.000246 | 0.000087 | 0.00020-0.00040 | 0.000375 | 0.000236 | 0.00020-0.00070 |
| Zn | 121 | 51 | 47-264 | 129 | 58 | 57.7-251.0 |
| Zr | 0.074 | 0.045 | 0.031-0.205 | 0.080 | 0.059 | 0.031-0.165 |

M – arithmetic mean, SD – standard deviation.

**Table 2.**Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in thyroid colloid goiter and adenoma

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Element | Thyroid tissue | | | | Ratio |
| Colloid goiter (CG) | Adenoma (TA) | Student’s t-test, *p*≤ | U-test, *p* | CG/TA |
| Ag | 0.192±0.038 | 0.181±0.050 | 0.861 | >0.05 | 1.06 |
| Al | 27.1±5.3 | 34.2±9.1 | 0.516 | >0.05 | 0.79 |
| Au | 0.0141±0.0030 | 0.0287±0.0110 | 0.247 | >0.05 | 0.49 |
| B | 5.50±3.8 | 3.38±1.12 | 0.598 | >0.05 | 1.63 |
| Be | 0.00072±0.00011 | 0.00181±0.00090 | 0.279 | >0.05 | 0.40 |
| Bi | 0.0585±0.0130 | 0.112±0.064 | 0.450 | >0.05 | 0.52 |
| Cd | 1.26±0.28 | 2.78±0.95 | 0.167 | >0.05 | 0.45 |
| Ce | 0.0186±0.0040 | 0.0246±0.0090 | 0.567 | >0.05 | 0.76 |
| Co | 0.0576±0.0049 | 0.0660±0.0135 | 0.571 | >0.05 | 0.87 |
| Cr | 1.18±0.24 | 1.36±0.24 | 0.596 | >0.05 | 0.87 |
| Cs | 0.0216±0.0050 | 0.052±0.038 | 0.467 | >0.05 | 0.42 |
| Er | 0.00299±0.00100 | 0.00400±0.00200 | 0.580 | >0.05 | 0.75 |
| Fe | 449±92 | 571±174 | 0.542 | >0.05 | 0.79 |
| Ga | 0.0210±0.0020 | 0.0223±0.0050 | 0.825 | >0.05 | 0.94 |
| Hg | 1.18±0.17 | 1.16±0.34 | 0.948 | >0.05 | 1.02 |
| La | 0.00990±0.00200 | 0.0116±0.0060 | 0.814 | >0.05 | 0.85 |
| Li | 0.0281±0.0030 | 0.0401±0.0100 | 0.275 | >0.05 | 0.70 |
| Mn | 1.77±0.23 | 1.67±0.54 | 0.875 | >0.05 | 1.06 |
| Mo | 0.183±0.026 | 0.233±0.055 | 0.429 | >0.05 | 0.79 |
| Nd | 0.0139±0.0020 | 0.0141±0.0030 | 0.948 | >0.05 | 0.99 |
| Ni | 2.63±0.54 | 3.95±1.39 | 0.406 | >0.05 | 0.67 |
| Pb | 0.94±0.41 | 1.86±1.24 | 0.503 | >0.05 | 0.51 |
| Pr | 0.00396±0.00100 | 0.00475±0.00200 | 0.695 | >0.05 | 0.83 |
| Rb | 9.50±0.50 | 8.96±0.82 | 0.815 | >0.05 | 1.06 |
| Sb | 0.127±0.019 | 0.140±0.034 | 0.749 | >0.05 | 0.91 |
| Sc | 0.0196±0.0060 | 0.0286±0.0140 | 0.552 | >0.05 | 0.69 |
| Se | 3.54±0.56 | 3.01±0.65 | 0.548 | >0.05 | 1.18 |
| Sm | 0.00169±0.00033 | 0.00252±0.00099 | 0.410 | >0.05 | 0.67 |
| Sn | 0.0458±0.0090 | 0.0756±0.0170 | 0.146 | >0.05 | 0.61 |
| Th | 0.0074±0.0010 | 0.0229±0.0011 | 0.214 | >0.05 | 0.32 |
| Tl | 0.00174±0.00021 | 0.00238±0.00067 | 0.391 | >0.05 | 0.73 |
| U | 0.00145±0.00022 | 0.00083±0.00020 | 0.077 | >0.05 | 1.75 |
| Y | 0.0113±0.0030 | 0.0115±0.0060 | 0.979 | >0.05 | 0.98 |
| Yb | 0.000246±0.000024 | 0.000375±0.000118 | 0.358 | >0.05 | 0.66 |
| Zn | 121±8 | 129±13 | 0.577 | >0.05 | 0.94 |
| Zr | 0.074±0.010 | 0.080±0.029 | 0.846 | >0.05 | 0.93 |

M – arithmetic mean, SEM – standard error of mean.

Published data on comparison of Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Fe, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Sc, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zrlevels in CG and TA were not found.

Thus, from obtained results it was possible to conclude that the common characteristics of CG and TA tissue samples in comparison with normal thyroid were elevated level of Ag, Al, Cr, Hg, Mn, Th, and Zn. Therefore, it is reasonable to conclude that these TE can be involved in etiology and pathogenesis of such thyroid disorders as CG and TA.

**Silver**

Ag is a TE with no recognized trace metal value in the human body [56]. 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 [57]. 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 [58]. In experimental studies it was shown that Ag nanoparticles may affect thyroid hormone metabolism [59]. More detailed knowledge of the Ag toxicity can lead to a better understanding of the impact on human health, including thyroid function.

**Table 3.**Comparison the trace element contents in different pathological transformations of thyroid

|  |  |  |  |
| --- | --- | --- | --- |
| Comparison with: | Normal thyroid\* | | Colloid Goiter |
| Element | ColloidGoiter | Adenoma | Adenoma |
| Ag | ↑ | ↑ | **=** |
| Al | ↑ | ↑ | **=** |
| Au | **=** | **=** | **=** |
| B | **=** | ↑ | **=** |
| Be | **=** | **=** | **=** |
| Bi | ↑ | **=** | **=** |
| Cd | ↓ | **=** | **=** |
| Ce | ↑ | **=** | **=** |
| Co | ↑ | **=** | **=** |
| Cr | ↑ | ↑ | **=** |
| Cs | **=** | **=** | **=** |
| Er | ↑ | **=** | **=** |
| Fe | ↑ | **=** | **=** |
| Ga | ↓ | **=** | **=** |
| Hg | ↑ | ↑ | **=** |
| La | ↑ | **=** | **=** |
| Li | ↑ | **=** | **=** |
| Mn | ↑ | ↑ | **=** |
| Mo | ↑ | **=** | **=** |
| Nd | ↑ | **=** | **=** |
| Ni | ↑ | **=** | **=** |
| Pb | **=** | **=** | **=** |
| Pr | ↑ | **=** | **=** |
| Rb | **=** | **=** | **=** |
| Sb | **=** | **=** | **=** |
| Sc | **=** | **=** | **=** |
| Se | ↑ | **=** | **=** |
| Sm | ↑ | **=** | **=** |
| Sn | ↓ | **=** | **=** |
| Th | **=** | **=** | **=** |
| Tl | ↑ | **=** | **=** |
| U | ↑ | **=** | **=** |
| Y | ↑ | **=** | **=** |
| Yb | **=** | **=** | **=** |
| Zn | ↑ | ↑ | **=** |
| Zr | **=** | **=** | **=** |

\* From analysis of previous publications [43,44],↑ - element content is higher, ↓ - element content is lower, **=** - no difference

**Aluminum**

Al is the most widely distributed metal in the environment. Environmental media may be contaminated by Al from anthropogenic sources and through the weathering of rocks and minerals [60]. The trace element Al is not described as essential, because no biochemical function has been directly connected to it. Toxic actions of Al induce oxidative stress, immunologic alterations, genotoxicity, and other disorders, including cell membrane perturbation, apoptosis, necrosis and dysplasia [60]. Furthermore, it was shown in experimental and epidemiological studies that Al can affect thyroid iodide uptake and hormones secretion [61,62].

**Chromium**

Cr-compounds are cytotoxic, genotoxic, and carcinogenic in nature. Some Cr forms, including hexavalent chromium (Cr6+), are toxicants known for their carcinogenic effect in humans. They have been classified as certain or probable carcinogens by the International Agency for Research on Cancer [63]. The lung cancer risk is prevalent in pigment chromate handlers, ferrochromium production workers, stainless steel welders, and chrome-platers [64]. Except in Cr-related industries and associated environments, Cr intoxication from environmental exposure is not common. However, it was found, that drinking water suppliesin many geographic areas contain chromium in the +3 and +6 oxidation states. Exposure of animals to Cr6+ in drinking water induced tumors in the mouse small intestine [65]. Many other animal experiments and in vitro studies demonstrate also that Cr can induce oxidative stress and exert cytotoxic effects [66]. Besides reactive oxygen species (ROS) generation, oxidative stress, and cytotoxic effects of Cr exposure, a variety of other changes like DNA damage, increased formation of DNA adducts and DNA-protein cross-links, DNA strand breaks, chromosomal aberrations and instability, disruption of mitotic cell division, chromosomal aberration, premature cell division, S or G2/M cell cycle phase arrest, and carcinogenesis also occur in humans or experimental test systems [64]. Recently, in acase-control study on the association of TE exposure and TBNs it was shown that Cr is a potential influencing factor for the risk of thyroid tumor and goiter [67].

**Mercury**

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, epidemiologic, and occupational studies of Hg in different chemical states shown significant alterations in thyroid hormones metabolism and thyroid gland parenchyma [67,69,70]. Moreover, Hg was classified as certain or probable carcinogen by the International Agency for Research on Cancer [[63](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5075591/#B1)]. For example, in Hg polluted area thyroid cancer incidence was almost 2 times higher than in adjacent control areas [71].

**Manganese**

Mn is an essential micronutrient because this TE acts as a co-factor in many enzymatic reactions involved in the metabolisms of lipid, protein, carbohydrate and amino acid, etc. [[72](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6616488/#B1-ijerph-16-02157)]. The diet, natural and anthropogenic contaminated environment are the main sources of Mn exposure in general populations. It was found in many experimental and epidemiologic studies that excessive environmental Mn exposure may affect the balance of thyroid hormone homeostasis via decreasing serum thyroid hormone levels, including T3 and T4[72]. Furthermore, recently, in a case-control study on the association of TE exposure and TBNs it was shown that Cr is a potential influencing factor for the risk of thyroid tumor and goiter [67].

**Thorium**

Th is a naturally radioactive TE, which effects by its chemical toxicity and radiation on skeleton, nervous and endocrine systems. The results of many experimental studies indicate that Th administration exerts hazardous effects on the neuroendocrine axis andcausesthe imbalance of thyroid hormones and structural changes in thyroid gland [73,74]. Moreover, an epidemiologic and clinicopathologic study found an apparentincreased prevalence of both benign and malignant thyroid disease in the group of patients treated with Th-contained compound (Thorotrast) [75].

**Zinc**

Zn, as a trace metal,has structural, catalytic and regulatory roles in normal and pathophysiology. This TE is a constituent of more than 3000 proteins and is a cofactor for over 300 enzymes [76]. Zn is an essential mediator of cell proliferation and differentiation through the regulation of DNA synthesis and mitosis. Zn also affects DNA repair pathways by regulating multiple intracellular signaling pathways and altering proteins involved in DNA maintenance [77]. This metal also maintenance the balance ofa cellular redox [78]. Thus, Zn is important cofactors in diverse cellular processes. Concern the thyroid function, Zn is involved in the synthesis of TSH and important for the proper functioning of T3 because T3 nuclear receptors contain Zn ions [79]. However, high Zn concentrations are toxic to the cells and the elevated level of Zn mass fractions in thyroid tissue may contribute to harmful effects on the gland. There are good reasons for such speculations since. experimental and epidemiological data support the hypothesis that Zn overload is a risk factor for benign and malignant tumors [77,80-82].

Characteristically, elevated or reduced levels of TE 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 TE in affected thyroid tissues researchers try to determine the role of the deficiency or excess of each TE in the etiology and pathogenesis of thyroid diseases. In our opinion, abnormal levels of many TE 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 TE level in pathologically altered tissue is the reason for alterations or vice versa.

**Limitations**

This study has several limitations. Firstly, analytical techniques employed in this study measure only fiftyTE(Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Fe, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Sc, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of TE investigated in normal thyroid and in pathologically altered tissue. Secondly, the sample size of CG group and, particularly, of TA group was relatively small and prevented investigations of TE contents in these groups using differentials like gender, histological types of CG and TA, nodules functional activity, stage of disease, and dietary habits of patients with CG and TA. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on TBNs-specific tissue Ag, Al, Cr, Hg, Mn, Th, and Zn level alteration and shows the necessity to continue TE research of TBNs.

Conclusion

In this work, TE analysis was carried out in the tissue samples of CG and TA using non-destructive analytical method INAA-LLR and destructive analytical method ICP-MS. It was shown that combination of these methods is an adequate analytical tool for the determination of fifty TE (Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Fe, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Sc, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr)content in the tissue samples of human thyroid in norm and pathology, including needle-biopsy specimens. It was observed that in both CG and TA tissues contents of Ag, Al, Cr, Hg, Mn, Th, and Zn increased, whereas levels of Au, Be, Cs, Pb, Rb, Sb, Sc, Th, Yb, and Zr did not changed in comparison with normal thyroid tissue.It was not found any differences between TE contents of CG and TA. From obtained results it was possible to conclude that the common characteristics of CG and TA tissue samples were elevated level of Ag, Al, Cr, Hg, Mn, Th, and Znin comparison with normal thyroid and, therefore, these TE can be involved in etiology and pathogenesis of such thyroid disorders as CG and TA.

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**Conflict of interest**

No conflict of interest associated with this work.

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**Author’s justification for comments**

**Remark K66**

1)…if it is possible to cite the route of access of each element in the human body whether it was oral or cutaneous ....etc

**Response to Reviewer.**All patients investigated in the study were inhabitants of environmentally sound (non-industrial and unpolluted) region(this sentence was added in text). It is well know, that in normal environmental conditions the human body receive with food about 90% chemical elements, 9% with drinking water, and 1% from air. However, there are some exclusions, for example, Ca and Mg in areas with hard water or I concentration in air near sea or ocean.

Anyway, some information on main sources of TE were added in text.

**Remark K66**

2) The author is demanded to cite the relationship between the distribution of elements in the periodic table and their effects.

**Response to Reviewer.**Excuse me, please, but it is impossible, because TE behaviour in organisms is very differ than in inanimate nature. Our knowledge on the topic is very short. For example, we can understand the competition between halogen I and such other halogens as Cl and Br, or the competition between Ca and rare earth elements because they have similar ion-radius. However, for example, Fe, Co, and Ni are transition metals of VIII groups of periodic table, but it is very difficult toexplain why Fe was chosen by blood hemoglobin, Co – by cobalamin, and Ni in more-more less concentration than Fe is a carcinogen.

**Remark K66**

3) You could have given details about which coexistenceof elements that worsen the epidemiological situation

**Response to Reviewer.**Excuse me, please, but I don’t understand whatever do you mean by that?

**Remark K66**

4) In the case of zinc, it’s known that zinc is the most important trace element for central nervous system. In such epidemiological cases. How you can explain that?

Are there any factors responsible for the misdistribution of the Zinc in the body?

**Response to Reviewer.**Zn is very important TE not only for central nervous system but for all systems of human body. Muscles and skeleton are the main pools of Zn in human body. The highest Zn concentration is in prostate, because Zn is used for prostate secret production. Best of my belief, we know nothing about mechanisms which regulate Zn distribution between organs. However, last decades Zn transporters in prostatic cells were found. Using prostate gland physiology as example, it is possible to understand that, similar the situation with I in thyroid,not only Zn deficiency, but overload of this element is very harmful. In developed countries there is no Zn deficiency in general population, but there is problem with Zn overload. The main source of Zn in human food is red meet and for the last century population consumption of red meet in developed countries increase in many times.

**Remark K71.** As the silver located in the same column (‘1B’ of the periodic table) as the gold and copper how it happened only silver induce such damage

If it is possible you can give more details ‘molecular explanation’

**Response to Reviewer.**

1. Ag concentration in human tissues and fluids at least order of magnitude higher, than Au concentration;
2. There are a few publications on the role of Cu excess in carcinogenesis;
3. There is nothing about ‘damage” in manuscript. It was only a statement of fact that Ag contents in thyroid hypertrophic and adenomatous tissue significantly higher than in normal gland.

**Remark K118**

Please follow the journal specifications for references. Please add DOI to articles if available. For example

Ishak AA, Alhadi AM, Al-Shamahy HA. Local experience of telemedicine: examples of cases in Yemen. Universal Journal of Pharmaceutical Research 2021; 6(1):34-37.  
<https://doi.org/10.22270/ujpr.v6i1.537>

**Response to Reviewer.**It was done.

**Remark K119**

Ref. 6 Zaichick V, IljinaT. Dietary iodine supplementation effect on the rat thyroid 131I blastomogenic action. In: Die Bedentung der Mengen- und Spurenelemente. 18. Arbeitstangung. Jena: Friedrich-Schiller-Universitat; 1998. p. 294-306.

Author has cited his own work in reference section. Author should replace/add others references. This is against the polices of the journal. There is a need of justification from the author for this.

**Response to Reviewer.**I was one of the first who found in experimental studies the iodine supplementation effect on blastomogenic action in thyroid. Now this fact was confirmed by many epidemiological studies. However, up to now in PubMed and Internet there is nothing about the experimental study results on the topic “iodine supplementation effect on blastomogenic action in thyroid”. Thus, it is impossible to add others references on the subject.

“Medical elementology” is a relative new direction of science. This direction is on the first phenomenological step of development. On the phenomenological step of development the main task is finding and collection the reliable results. When the collection of reliable results will be quite enough, more deep interpretations of results could be available.

I agree that you have raised several valid, most interesting and important points in your comments on my work. However I respectfully suggest that the present work contains sufficient new material to allow for publication in its corrected version.

Thank you again very much for the time and trouble you took to review this paper.

Kind regards.

Vladimir Zaichick