**Title**: Age dependence of iodine to some trace element content ratios in normal thyroid of males

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Age dependence of iodine to some trace element content ratios in normal thyroid of males

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**Abstract**

**Background**: Thyroid diseases rank second among endocrine disorders, and prevalence of the diseases is higher in the elderly as compared to the younger population. An excess or deficiency of trace element (TE) contents in thyroid play important role in goitro- and carcinogenesis of gland. **Methods**: The correlations between age and thirty-eight TE, including I, as well as between age and I/TE content ratios in normal thyroid of 72 males (age range 2-80 years) was investigated by two methods: instrumental neutron activation analysis and inductively coupled plasma mass spectrometry.

**Results**: Our data reveal that the Cd, I and Se contents, as well as the I/B, I/Be, I/Cr, I/Ga, I/Li, I/Mn, I/Rb, I/Tb, I/U, and I/Y content ratios increase, while B, Dy, Ga, Mn, U, Y contents and I/Cd and I/Gd content ratios decrease in the normal thyroid of male during a lifespan.

**Conclusions**: A goitrogenic and tumorogenic effect at least of excessive Cd and inadequate Cr and Mn levels in the thyroid of males with increasing age may be assumed.

**Keywords**: thyroid; trace elements; age-related changes; neutron activation analysis; inductively coupled plasma mass spectrometry

**Introduction**

According to the World Health Organization (WHO), thyroid diseases rank second among endocrine disorders after diabetes mellitus. More than 665 million people in the world have endemic goiter or suffer from other thyroid pathologies. At the same time, according to the same statistics, the increase in the number of thyroid diseases in the world is 5% per year [1]. It has been suggested that risk factors for the development of thyroid disorders may be numerous factors, including genetics, radiation, autoimmune diseases, as well as adverse environmental factors, such as an increase in the content of various chemicals in the environment [2].

Trace elements (TE) are among these various chemicals, because their levels in the environment have increased significantly over the past hundred years as a result of the industrial revolution and the tremendous technological changes that have taken place in metallurgy, chemical production, electronics, agriculture, food processing and storage, cosmetics, pharmaceuticals and medicine. In connection with these changes, the levels and ratio of TE entering the human body from the outside have been significantly disturbed, compared with the conditions in which human societies have lived for many millennia.

More than 50 years ago, we formulated the postulate about the somatic TE homeostasis, which is now generally recognized [3]. According to this postulate, under evolutionary environmental conditions, the mechanisms of homeostasis of organisms maintain the levels and ratios of TE in tissues and organs within certain limits. If the content of TE in the environment changes significantly, the mechanisms of somatic homeostasis may respond inadequately. Inadequate response of homeostasis mechanisms leads to changes in TE levels in tissues and organs, which, in turn, can affect their function and lead to the development of pathological conditions. The correctness of this conclusion was illustrated by us earlier on the example of the study of the role of TE in the normal and pathophysiology of the prostate [4-24]. It was shown, in particular, that a special role in the development of pathological transformations of the prostate is played by disturbances in the relationship between TE in the tissue and gland secretion. Moreover, it was found that changes in the relationship between TE can be used as highly informative markers of various prostate diseases, including malignant tumors [25-40]. These findings stimulated our investigations of TE relationships in thyroid tissue in normal and pathological conditions.

There are many studies regarding TE content in human thyroid, using chemical techniques and instrumental methods [41-64]. However, among the published data, no works on the relationship of TE in the normal human thyroid were found.

This work had three aims. The primary purpose of this study was to determine reliable values for the Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Ga, Gd, Hg, I, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Ti, Tl, U, Y, and Zn mass fractions in the normal thyroid of subjects ranging from children to elderly males using instrumental neutron activation analysis (INAA) combined in consecutive order with destructive inductively coupled plasma mass spectrometry (ICP-MS) and calculate individual values of I/Ag, I/Al, I/B, I/Be, I/Bi, I/Cd, I/Ce, I/Co, I/Cr, I/Cs, I/Dy, I/Er, I/Fe, I/Ga, I/Gd, I/Hg, I/La, I/Li, I/Mn, I/Mo, I/Nb, I/Nd, I/Ni, I/Pb, I/Pr, I/Rb, I/Sb, I/Sc, I/Se, I/Sm, I/Sn, I/Tb, I/Ti, I/Tl, I/U, I/Y, and I/Zn. The second aim was to compare thirty-eight TE mass fractions in thyroid gland obtained in the study with published data. The final aim was to estimate the correlations between age and TE contents, as well as between age and I/TE content ratios in normal thyroid of males.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, 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.

**Materials and Methods**

**Thyroid tissue samples**

Randomly selected tissue samples of the thyroid gland were obtained from autopsies of 72 practically healthy residents (European-Caucasian nationality) of the Obninsk city, who died suddenly. The age of the deceased males ranged from 2 to 80 years. The main causes of sudden death were injuries in car accidents and trauma. Several males have died from suicide, alcohol poisoning, stroke, acute heart failure, and pulmonary embolism. Autopsies were carried out in the forensic medical examination department of the city hospital. In the anamnesis of the deceased males there were no chronic diseases, as well as medications or nutritional supplements that affect the development and function of the thyroid gland,

Thyroid tissue samples were taken from the right lobe of the gland using a titanium scalpel [65] and divided into two parts. One part was subjected to histological examination in order to confirm compliance with the age norm, as well as to exclude the presence of microadenomas and latent cancer. The second part was intended to determine the content of TE in it.

**Methods**

Thyroid tissue samples were delivered frozen to the Medical Radiological Research Center, where they were weighed and stored at -20°C. Subsequently, all samples were lyophilized and homogenized [66]. To determine the contents of the TE by comparison with a known standard, aliquots of commercial, chemically pure compounds were used [67]. Ten subsamples of the Certified Reference Material (CRM) produced by the International Atomic Energy Agency (IAEA) IAEA H-4 (Animal Muscle) and IAEA HH-1 (Human Hair), as well as Polish CRM INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs were analyzed to estimate the precision and accuracy of results. The CRM subsamples were prepared in the same way as the samples of dry homogenized thyroid tissue.

The content of I was determined by INAA using short irradiation in a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor in Obninsk. The neutron flux in the channel was 1.7 × 1013n cm−2 s−1. A vertical channel of nuclear reactor WWR-c with a neutron flux of 1.3×1013 n×cm-2×s-1 was applied to determine the content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by long irradiation. Details of sample preparation and used nuclear reactions, induced radionuclides, gamma-energies and semiconductor spectrometry were presented in our earlier publications concerning TE contents in human scalp hair [68,69]. After non-destructive INAA investigation the thyroid samples were decomposed in autoclaves and used for ICP-MS. The content of Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Ga, Gd, Hg, I, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Se, Sm, Sn, Tb, Ti, Tl, U, Y, and Zn was determined by ICP-MS using an ICP-MS Thermo-Fisher “X-7” Spectrometer (Thermo Electron, USA). The TE concentrations in aqueous solutions were determined by the quantitative method using multi elemental calibration solutions ICP-MS-68A and ICP-AM-6-A produced by High-Purity Standards (Charleston, SC 29423, USA). Indium was used as an internal standard in all measurements. Information detailing with the ICP-MS methods used and other details of the analysis was presented in our previous publication concerning TE contents in human prostate [70-73].

**Computer programs and statistics**

A dedicated computer program for INAA mode optimization was used [74]. All thyroid samples were prepared in duplicate, and mean values of TE contents were used in final calculation. For TE whose content was determined by two methods, the average value was calculated. The main statistical characteristics of the TE content and the I/TE content ratio of. such as the arithmetic mean, standard deviation, standard error of the mean, minimum and maximum values, median, percentiles with levels of 0.025 and 0.975 were found using Microsoft Office Excel. Pearson's correlation coefficient was used in Microsoft Office Excel to calculate the relationship "age – TE mass fraction" and "age – I/TE mass fraction”.

**Results**

Table 1 depicts the similarity of the means of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions in the normal thyroid of male determined by both INAA and ICP-MS methods.

**Table 1.** Comparison of the mean values (M±SEM) of the chemical element mass fractions (mg/kg, on dry tissue) in the normal thyroid of males obtained by both INAA and ICP-MS methods

|  |  |  |  |
| --- | --- | --- | --- |
| Element | INAA  M1 | ICP-MS  M2 | ∆, % |
| Ag | 0.0156±0.0021 | 0.0121±0.0018 | 22.4 |
| Co | 0.0352±0.0031 | 0.0334±0.0032 | 5.1 |
| Cr | 0.520±0.041 | 0.427±0.039 | 17.9 |
| Fe | 222±12 | 223±15 | -0.5 |
| Hg | 0.0437±0.0048 | 0.0970±0.0102 | -122 |
| Rb | 7.89±0.58 | 8.38±0.61 | -6.2 |
| Sb | 0.108±0.010 | 0.0705±0.0097 | 34.7 |
| Se | 2.36±0.17 | 2.10±0.17 | 11.0 |
| Zn | 103±5.5 | 95.4±5.1 | 7.4 |

M – arithmetic mean, SEM – standard error of mean, ∆=[(M1 – M2)/M1] ∙100%.

Tables 2 and 3 represents the main statistical characteristics of the Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Ga, Gd, Hg, I, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Ti, Tl, U, Y, Zn mass fractions and of the I/Ag, I/Al, I/B, I/Be, I/Bi, I/Cd, I/Ce, I/Co, I/Cr, I/Cs, I/Dy, I/Er, I/Fe, I/Ga, I/Gd, I/Hg, I/La, I/Li, I/Mn, I/Mo, I/Nb, I/Nd, I/Ni, I/Pb, I/Pr, I/Rb, I/Sb, I/Sc, I/Se, I/Sm, I/Sn, I/Tb, I/Ti, I/Tl, I/U, I/Y, and I/Zn mass fraction ratios in normal thyroid of males, respectively.

The comparison of our results with published data for contents of all TE in the human thyroid determined in the present study is shown in Table 4.

Pearson's correlation coefficients in Tables 5 and 6 estimate the effect of age on the TE contents and I/TE content ratios, respectively.

**Table 2**. Some statistical parameters of thirty-eight trace element mass fraction (mg/kg, dry tissue) in the normal thyroid of male

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Element | M | SD | SEM | Min | Max | Median | P 0.025 | P 0.975 |
| Ag | 0.0133 | 0.0122 | 0.0017 | 0.0017 | 0.0789 | 0.0088 | 0.00193 | 0.0321 |
| Al | 11.3 | 14.9 | 2.3 | 0.800 | 69.3 | 6.4 | 1.12 | 58.6 |
| B | 0.491 | 0.473 | 0.071 | 0.200 | 2.30 | 0.300 | 0.200 | 2.03 |
| Be | 0.00048 | 0.00052 | 0.00008 | 0.00010 | 0.00240 | 0.00025 | 0.00010 | 0.00170 |
| Bi | 0.00386 | 0.00511 | 0.00079 | 0.00030 | 0.0245 | 0.00230 | 0.00050 | 0.0217 |
| Cd | 2.22 | 2.13 | 0.33 | 0.107 | 8.26 | 1.41 | 0.155 | 7.95 |
| Ce | 0.00773 | 0.00817 | 0.00123 | 0.00100 | 0.0348 | 0.00435 | 0.00123 | 0.0312 |
| Co | 0.0345 | 0.0238 | 0.0031 | 0.0100 | 0.127 | 0.0260 | 0.0124 | 0.101 |
| Cr | 0.474 | 0.265 | 0.038 | 0.130 | 1.30 | 0.400 | 0.152 | 0.984 |
| Cs | 0.0263 | 0.0177 | 0.0026 | 0.0113 | 0.0924 | 0.0210 | 0.0116 | 0.0806 |
| Dy | 0.00108 | 0.00118 | 0.00018 | 0.00030 | 0.00600 | 0.00060 | 0.00030 | 0.00367 |
| Er | 0.00034 | 0.00028 | 0.00004 | 0.00010 | 0.00110 | 0.00027 | 0.00010 | 0.00110 |
| Fe | 222 | 87 | 11 | 52.0 | 474 | 224 | 72.8 | 406 |
| Ga | 0.0318 | 0.0140 | 0.0022 | 0.0100 | 0.0700 | 0.0300 | 0.0100 | 0.0697 |
| Gd | 0.00092 | 0.00079 | 0.00012 | 0.00040 | 0.00470 | 0.00060 | 0.00040 | 0.00219 |
| Hg | 0.0610 | 0.0394 | 0.0055 | 0.0090 | 0.151 | 0.0490 | 0.0105 | 0.150 |
| I | 1486 | 902 | 130 | 220 | 3744 | 1337 | 222 | 3443 |
| La | 0.00454 | 0.00485 | 0.00074 | 0.00040 | 0.0219 | 0.00250 | 0.00040 | 0.0188 |
| Li | 0.0225 | 0.0168 | 0.0028 | 0.00400 | 0.0977 | 0.0179 | 0.00463 | 0.0547 |
| Mn | 1.27 | 0.47 | 0.06 | 0.470 | 2.30 | 1.16 | 0.534 | 2.21 |
| Mo | 0.0856 | 0.0428 | 0.0064 | 0.0305 | 0.299 | 0.0804 | 0.0388 | 0.156 |
| Nb | 0.584 | 0.952 | 0.145 | 0.0130 | 3.77 | 0.142 | 0.0130 | 3.42 |
| Nd | 0.00388 | 0.00312 | 0.00047 | 0.00020 | 0.0139 | 0.00295 | 0.00081 | 0.0131 |
| Ni | 0.467 | 0.375 | 0.056 | 0.0740 | 1.80 | 0.345 | 0.120 | 1.48 |
| Pb | 0.242 | 0.271 | 0.040 | 0.0260 | 1.60 | 0.170 | 0.0450 | 0.794 |
| Pr | 0.00102 | 0.00080 | 0.00012 | 0.00010 | 0.00350 | 0.00070 | 0.00021 | 0.00345 |
| Rb | 8.07 | 3.96 | 0.50 | 3.53 | 22.6 | 7.10 | 3.82 | 18.5 |
| Sb | 0.0895 | 0.0705 | 0.0091 | 0.00470 | 0.308 | 0.0662 | 0.00954 | 0.291 |
| Sc | 0.0390 | 0.0359 | 0.0085 | 0.00050 | 0.0860 | 0.0344 | 0.00093 | 0.0860 |
| Se | 2.23 | 1.28 | 0.17 | 0.530 | 5.80 | 1.71 | 0.810 | 5.64 |
| Sm | 0.00049 | 0.00047 | 0.00007 | 0.00010 | 0.00210 | 0.00034 | 0.00010 | 0.00150 |
| Sn | 0.0674 | 0.0556 | 0.0085 | 0.00900 | 0.211 | 0.0507 | 0.00912 | 0.199 |
| Tb | 0.00020 | 0.00011 | 0.00002 | 0.00010 | 0.00050 | 0.00015 | 0.00010 | 0.00042 |
| Ti\* | 3.43 | 3.36 | 0.51 | 0.530 | 14.5 | 2.25 | 0.908 | 11.9 |
| Tl | 0.00099 | 0.00053 | 0.00008 | 0.00029 | 0.00290 | 0.00092 | 0.00030 | 0.00219 |
| U | 0.00040 | 0.00033 | 0.00005 | 0.00010 | 0.00140 | 0.00030 | 0.00010 | 0.00111 |
| Y | 0.00247 | 0.00221 | 0.00034 | 0.00100 | 0.0100 | 0.00160 | 0.00100 | 0.00818 |
| Zn | 99.1 | 39.4 | 5.0 | 34.0 | 215 | 92.5 | 44.0 | 200 |

M – arithmetic mean, SD – standard deviation, SEM – standard error of mean, Min – minimum value, Max – maximum value, P 0.025 – percentile with 0.025 level, P 0.975 – percentile with 0.975 level.

**Discussion**

A good agreement of our results for the Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Ga, Gd, Hg, I, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Ti, Tl, U, Y, Zn mass fractions with the certified values of CRM IAEA H-4 (Animal Muscle), IAEA HH-1 (Human Hair), INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs [68-73] as well as the similarity of the means of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions in the normal thyroid of male determined by both INAA and ICP-MS methods (Table 1) demonstrates an acceptable precision and accuracy of the results obtained in the study and presented in Tables 2-6.

The content of TE was determined in all or most of the examined samples, which made it possible to calculate the main statistical parameters: the mean value of the mass fraction (M), standard deviation (SD), standard error of the mean (SEM), minimum (Min), maximum (Max), median (Med), and percentiles with levels of 0.025 (P 0.025) and 0.975 (P 0.975), of the Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Ga, Gd, Hg, I, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Ti, Tl, U, Y, and Zn mass fractions (Table 2), as well as I/Ag, I/Al, I/B, I/Be, I/Bi, I/Cd, I/Ce, I/Co, I/Cr, I/Cs, I/Dy, I/Er, I/Fe, I/Ga, I/Gd, I/Hg, I/La, I/Li, I/Mn, I/Mo, I/Nb, I/Nd, I/Ni, I/Pb, I/Pr, I/Rb, I/Sb, I/Sc, I/Se, I/Sm, I/Sn, I/Tb, I/Ti, I/Tl, I/U, I/Y, and I/Zn mass fraction ratios (Table 3) in normal thyroid of males.

**Table 3**. Some statistical parameters of iodine/trace element mass fraction ratios in the normal thyroid of male

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ratio | M | SD | SEM | Min | Max | Median | P 0.025 | P 0.975 |
| I/Ag | 246629 | 216677 | 32665 | 6471 | 916842 | 211435 | 10343 | 827405 |
| I/Al | 463 | 552 | 91 | 5.14 | 2381 | 226 | 8.43 | 1647 |
| I/B | 5492 | 4696 | 772 | 367 | 17855 | 3753 | 585 | 17365 |
| I/Be | 6561448 | 5767441 | 948161 | 448980 | 26480000 | 5905000 | 592998 | 18717500 |
| I/Bi | 1493340 | 2299893 | 388753 | 24857 | 11903333 | 703750 | 33890 | 7670900 |
| I/Cd | 1899 | 3033 | 513 | 83.0 | 16131 | 903 | 90.7 | 10606 |
| I/Ce | 478036 | 433014 | 72169 | 10776 | 1700476 | 328906 | 22435 | 1320005 |
| I/Co | 67455 | 47817 | 6831 | 4795 | 185563 | 55192 | 8037 | 156533 |
| I/Cr | 4523 | 4017 | 627 | 373 | 17005 | 3300 | 481 | 15817 |
| I/Cs | 89590 | 65173 | 10714 | 4575 | 263522 | 73466 | 7170 | 237534 |
| I/Dy | 3101253 | 2720084 | 447179 | 81481 | 11903333 | 2235000 | 197148 | 523560 |
| I/Er | 8701775 | 8954255 | 1472070 | 252874 | 35710000 | 6600000 | 523560 | 30742000 |
| I/Fe | 10.6 | 10.0 | 1.4 | 0.60 | 56.8 | 7.39 | 0.92 | 31.8 |
| I/Ga | 61739 | 46655 | 7670 | 3929 | 192333 | 56460 | 7818 | 155088 |
| I/Gd | 2703471 | 2175673 | 357678 | 129412 | 8927500 | 2235000 | 286991 | 7541500 |
| I/Hg | 48998 | 55726 | 8703 | 2529 | 262813 | 28085 | 2840 | 172417 |
| I/La | 824982 | 820629 | 136772 | 12055 | 4237500 | 612242 | 36507 | 2265590 |
| I/Li | 125546 | 129183 | 23202 | 7881 | 631702 | 101604 | 8150 | 391353 |
| I/Mn | 1675 | 1415 | 206 | 200 | 7066 | 1334 | 234 | 5469 |
| I/Mo | 23271 | 16379 | 2693 | 2037 | 58735 | 22309 | 2624 | 52175 |
| I/Nb | 32697 | 58411 | 9735 | 101 | 274692 | 8225 | 168 | 178039 |
| I/Nd | 801763 | 680477 | 113413 | 37183 | 2483333 | 559049 | 53536 | 2433021 |
| I/Ni | 5651 | 5149 | 847 | 327 | 23807 | 4566 | 358 | 15741 |
| I/Pb | 15303 | 16460 | 2706 | 381 | 67698 | 9500 | 668 | 56214 |
| I/Pr | 2708883 | 2033190 | 338865 | 105600 | 7117500 | 2255051 | 204200 | 6948188 |
| I/Rb | 270 | 211 | 29 | 14.5 | 855 | 220 | 27.3 | 767 |
| I/Sb | 28616 | 26801 | 3753 | 2828 | 132553 | 21675 | 3502 | 93842 |
| I/Sc | 384636 | 528810 | 132202 | 3671 | 1588000 | 73102 | 3973 | 1456287 |
| I/Se | 971 | 765 | 107 | 96.0 | 3708 | 767 | 137 | 3095 |
| I/Sm | 7487096 | 8350864 | 1411554 | 240000 | 35710000 | 4718333 | 381100 | 29556000 |
| I/Sn | 48769 | 46803 | 7801 | 1440 | 182479 | 34351 | 3296 | 165956 |
| I/Tb | 11312807 | 8360862 | 1374517 | 523810 | 34620000 | 9703333 | 1148581 | 30057000 |
| I/Ti\* | 978 | 876 | 146 | 18.0 | 3858 | 811 | 49.5 | 2714 |
| I/Tl | 2723510 | 2573438 | 423071 | 146667 | 11159375 | 2104545 | 173067 | 9213211 |
| I/U | 7863331 | 8034124 | 1320802 | 273636 | 35710000 | 5646000 | 318540 | 27403000 |
| I/Y | 1150425 | 962705 | 158268 | 36125 | 3571000 | 1013333 | 58423 | 3472900 |
| I/Zn | 21.0 | 13.4 | 1.8 | 2.03 | 63.4 | 21.7 | 2.44 | 45.7 |

M – arithmetic mean, SD – standard deviation, SEM – standard error of mean, Min – minimum value, Max – maximum value, P 0.025 – percentile with 0.025 level, P 0.975 – percentile with 0.975 level, \*scalpel made od Ti was used in sample preparation

The values ​​of M, SD, and SEM can be used to compare data for different groups of samples only under the condition of a normal distribution of the results of determining the content of TE in the samples under study. Statistically reliable identification of the law of distribution of results requires large sample sizes, usually several hundred samples, and therefore is rarely used in biomedical research. In the conducted study, we could not prove or disprove the “normality” of the distribution of the results obtained due to the insufficient number of samples studied. Therefore, in addition to the M, SD, and SEM values, such statistical characteristics as median, range (Min-Max) and percentiles P 0.025 and P 0.975 were calculated, which are valid for any law of distribution of the results of TE content in thyroid tissue.

**Table 4.**Median, minimum and maximum value of means of trace element contents in the normal thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Element | Published data [Reference] | | | This work |
| Median of means  (n)\* | Min of means  M or M±SD, (n)\*\* | Max of means  M or M±SD, (n)\*\* | M±SD |
| Ag | 0.25 (12) | 0.000784 (16) [41] | 1.20±1.24 (105) [42] | 0.0133±0.0122 |
| Al | 33.6 (12) | 0.33 (-) [43] | 420 (25) [44] | 11.3±14.9 |
| B | 0.151 (2) | 0.084 (1) [49] | 0.46 (1) [49] | 0.491±0.473 |
| Be | 0.042 (3) | 0.000924(16) [41] | <0.12 (-) [48] | 0.00048±0.00052 |
| Bi | 0.126 (4) | 0.0339 (16) [41] | <0.4 (-) [48] | 0.00386±0.00511 |
| Cd | 1.68 (20) | 0.12 (131) [45] | 47.6±8.0 (16) [50] | 2.22±2.13 |
| Ce | 0.22 (1) | 0.22 (59) [41] | 0.22 (59) [41] | 0.00773±0.00817 |
| Co | 0.306 (25) | 0.016 (66) [51] | 70.4±40.8 (14) [52] | 0.0345±0.0238 |
| Cr | 0.69 (17) | 0.088 (83) [53] | 24.8±2.4 (4) [46] | 0.474±0.265 |
| Cs | 0.066 (6) | 0.0112±0.0109 (14) [54] | 0.109±0.370 (48) [55] | 0.0263±0.0177 |
| Dy | 0.00106 (1) | 0.00106 (60) [41] | 0.00106 (60) [41] | 0.00108±0.00118 |
| Er | 0.00068 (1) | 0.00068 (60) [41] | 0.00068 (60) [41] | 0.00034±0.00028 |
| Fe | 252 (21) | 56 (120)  [56] | 3360 (25) [44] | 222±87 |
| Ga | 0.273 (3) | <0.04 (-) [48] | 1.7±0.8 (-) [57] | 0.0318±0.0140 |
| Gd | 0.00256 (1) | 0.00256 (59) [41] | 0.00256 (59) [41] | 0.00092±0.00079 |
| Hg | 0.08 (13) | 0.0008±0.0002 (10)  [47] | 396±40 (4) [46] | 0.0610±0.0394 |
| I | 1888 (95) | 159±8 (23) [58] | 5772±2708 (50) [59] | 1486±902 |
| La | 0.068 (3) | 0.052 (59) [41] | <4.0 (-) [48] | 0.00454±0.00485 |
| Li | 6.3 (2) | 0.092 (-) [48] | 12.6 (180) [60] | 0.0225±0.0168 |
| Mn | 1.62 (40) | 0.076 (83) [53] | 69.2±7.2 (4) [46] | 1.27±0.47 |
| Mo | 0.40 (11) | 0.0288±0.0096 (39) [47] | 516±292 (14) [52] | 0.0856±0.0428 |
| Nb | <4.0 (1) | <4.0 (-) [48] | <4.0 (-) [48] | 0.584±0.952 |
| Nd | 0.0108 (1) | 0.0108 (60) [41] | 0.0108 (60) [41] | 0.00388±0.00312 |
| Ni | 0.44 (19) | 0.0084 (83) [53] | 33.6±3.6 (4) [46] | 0.467±0.375 |
| Pb | 0.58 (25) | 0.021 (83) [53] | 68.8±6.8 (4) [46] | 0.242±0.271 |
| Pr | 0.0034 (1) | 0.0034 (59) [41] | 0.0034 (59) [41] | 0.00102±0.00080 |
| Rb | 7.8 (9) | ≤0.85 (29) [47] | 294±191 (14) [52] | 8.07±3.96 |
| Sb | 0.15 (10) | 0.040±0.003 (-) [61] | ≤12.4 (-) [48] | 0.0895±0.0705 |
| Sc | 0.009 (4) | 0.0018±0.0003 (17) [62] | 0.0135±0.0045 (10) [47] | 0.0390±0.0359 |
| Se | 2.32 (21) | 0.436 (40) [63] | 756±680 (14) [52] | 2.23±1.28 |
| Sm | 0.00216 (1) | 0.00216 (60) [41] | 0.00216 (60) [41] | 0.00049±0.00047 |
| Sn | 0.67 (7) | 0.0235 (16) [41] | -≤3.8 (17) [64] | 0.0674±0.0556 |
| Tb | 0.000224 (1) | 0.000224 (60) [41] | 0.000224 (60) [41] | 0.00020±0.00011 |
| Ti | 1.42 (8) | 0.084 (83) [53] | 73.6±7.2 (4) [46] | 3.43±3.36 |
| Tl | <0.2 (2) | 0.00138 (16) [41] | <0.4 (-) [48] | 0.00099±0.00053 |
| U | 0.0060 (11) | 0.00014 (66) [51] | 0.428±0.143 (10) [47] | 0.00040±0.00033 |
| Y | <2.9 (2) | 0.00225 (16) [51] | ≤5.9 (17) [64] | 0.00247±0.00221 |
| Zn | 110 (56) | 2.1 (-) [43] | 820±204 (14) [52] | 99.1±39.4 |

M –arithmetic mean, SD – standard deviation, Min – minimum, Max – maximum, (n)\* – number of all references, (n)\*\* – number of samples.

Values obtained for Al, B, Cd, Cr, Cs, Dy, Er, Fe, Gd, Hg, Mn, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Tb, Ti, and Zn contents in the normal human thyroid (Table 4) agree well with median of mean values reported by other researches [41-64]. The obtained means for Ag, Co, Mo, Sn, Y, and U were almost one-three orders of magnitude lower median of previously reported means but inside the range of means (Table 4). The mean obtained for Be, Bi, Ce, Ga, La, Li, and Tl were also one-three orders of magnitude lower than the median of previously reported data and outside the range of previously reported means (under a minimal value of published means).

In some published articles, the values of the mass fractions of TE were presented in terms of ash or wet mass of the thyroid tissue. Therefore, we recalculated these data for dry mass basis using published values of 75% for water [55] and 4.16% for ash [75] in adult thyroids. No published data referring to I/Ag, I/Al, I/B, I/Be, I/Bi, I/Cd, I/Ce, I/Co, I/Cr, I/Cs, I/Dy, I/Er, I/Fe, I/Ga, I/Gd, I/Hg, I/La, I/Li, I/Mn, I/Mo, I/Nb, I/Nd, I/Ni, I/Pb, I/Pr, I/Rb, I/Sb, I/Sc, I/Se, I/Sm, I/Sn, I/Tb, I/Ti, I/Tl, I/U, I/Y, and I/Zn mass fraction ratios in human thyroid was found.

**Table 5.** Correlations between age (years) and trace element mass fractions (mg/kg, dry mass basis) in the normal thyroid of male (*r* – coefficient of correlation)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Element | Ag | Al | B | Be | Bi | Cd | Ce | Co | Cr | Cs |
| *r* | -0.03 | -0.29 | -0.37a | -0.29 | -0.11 | 0.72c | 0.03 | -0.13 | -0.13 | 0.05 |
| Element | Dy | Er | Fe | Ga | Gd | Hg | I | La | Li | Mn |
| *r* | -0.35a | -0.09 | -0.14 | -0.37a | -0.24 | 0.09 | 0.32b | 0.12 | -0.31 | -0.35b |
| Element | Mo | Nb | Nd | Ni | Pb | Pr | Rb | Sb | Sc | Se |
| *r* | -0.03 | -0.17 | 0.04 | -0.18 | -0.10 | 0.10 | -0.17 | -0.01 | -0.03 | 0.42c |
| Element | Sm | Sn | Tb | Ti | Tl | U | Y | Zn |  |  |
| *r* | -0.22 | 0.18 | -0.31 | 0.01 | 0.02 | -0.46b | -0.33a | -0.02 |  |  |

Statistically significant values: a *p*≤0.05, b *p*≤0.01, c *p*≤0.001.

**Table 6.** Correlations between age (years) and iodine/trace element mass fraction ratios in the normal thyroid of male (*r* – coefficient of correlation)

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Element | I/Ag | I/Al | I/B | I/Be | I/Bi | I/Cd | I/Ce | I/Co | I/Cr | I/Cs |
| *r* | -0.07 | 0.26 | 0.50b | 0.38a | 0.26 | -0.38a | 0.01 | 0.11 | 0.35a | 0.20 |
| Element | I/Dy | I/Er | I/Fe | I/Ga | I/Gd | I/Hg | I/La | I/Li | I/Mn | I/Mo |
| *r* | 0.34 | 0.24 | 0.25 | 0.35a | -0.38a | -0.02 | -0.06 | 0.33a | 0.37a | 0.14 |
| Element | I/Nb | I/Nd | I/Ni | I/Pb | I/Pr | I/Rb | I/Sb | I/Sc | I/Se | I/Sm |
| *r* | 0.16 | -0.18 | 0.20 | 0.11 | 0.01 | 0.31a | 0.11 | 0.10 | -0.19 | 0.23 |
| Element | I/Sn | I/Tb | I/Ti | I/Tl | I/U | I/Y | I/Zn |  |  |  |
| *r* | -0.14 | 0.39a | 0.26 | 0.23 | 0.34a | 0.39a | 0.17 |  |  |  |

Statistically significant values: a *p*≤0.05, b *p*≤0.01.

With age, the Cd, I and Se contents increase, while B, Dy, Ga, Mn, U and Y contents decrease (Table 5). If we consider the age-related changes in TE in relation to the content of iodine in the thyroid gland, which increases with age, then it can be noted that the I/Cd and I/Gd ratios decrease, while the I/B, I/Be, I/Cr, I/Ga, I/Li, I/Mn, I/Rb, I/Tb, I/U, and I/Y ratios increase. A decrease in the I/Cd ratio indicates that the accumulation of Cd with age is so significant that it is not compensated by an age-related increase in the level of I in the thyroid gland of men. On the other hand, for example, an increase in ratios such as I/Cr and I/Mn reveals an age-related deficiency of Cr and Mn in relation to iodine level. It should be noted that the ratio of such important elements for the function of the thyroid gland as I and Se does not change with age, in other words, the age-related increase in the content of I in the thyroid gland of men is accompanied by an adequate increase in the content of Se.

Previously, it was shown that an increase in the content of such TE as Cd in the tissues of various organs can lead not only to disruption of their normal functioning, but also be the cause of the development of various pathological conditions, including malignant tumors [20]. Deficiency of such intensely involved in the biochemical processes of the body TE as Cr and Mn can also be the cause of pathological conditions of various tissues and organs. including the thyroid gland [3,8]. Thus, in the present study was found multidirectional age-related changes in at least such TEs as Cd, on the one hand, and Cr and Mn. on the other hand, which may be responsible for the increase in the incidence of goiter and thyroid cancer with increasing age of males.

**Conclusion**

The combination of INAA and ICP-MS is a useful analytical tool for the determination of TE contents in the thyroid tissue samples. This method makes it possible to determine the content of at least thirty-eight TE.

Our data reveal that the Cd, I and Se contents, as well as the I/B, I/Be, I/Cr, I/Ga, I/Li, I/Mn, I/Rb, I/Tb, I/U, and I/Y content ratios increase, while B, Dy, Ga, Mn, U, Y contents and I/Cd and I/Gd content ratios decrease in the normal thyroid of male during a lifespan. Therefore, a goitrogenic and tumorogenic effect at least of excessive Cd and inadequate Cr and Mn levels in the thyroid of males with increasing age may be assumed.

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