Association between Age and Twenty Chemical Element Contents in Intact Thyroid of Males

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Abstract

The prevalence of thyroid dysfunction is higher in the older adults as compared to the younger population. An excess or deficiency of chemical element contents in thyroid plays an important role in goitro- and carcinogenesis of gland. The variation with age of the mass fraction of twenty chemical elements in intact thyroid of 72 males (mean age 37.8 years, range 2-80 years) was investigated. Measurements were performed using neutron activation analysis and inductively coupled plasma atomic emission spectrometry. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for chemical element analysis. This work revealed that there is a statistically significant increase in Ca and I mass fraction, as well as a decrease in Al, B, Ba, K, Li, Mn, and P mass fraction in the normal thyroid of male during a lifespan. Results of the study showed that for older males there is a goitrogenic and carcinogenic association with inadequate levels of the thyroid parenchymal chemical elements as Al, B, Ba, Ca, K, Li, Mn, and P, some of which can increase intra-thyroid oxidative stress.

Introduction

The endocrine organs, including the thyroid gland, undergo important functional changes during aging and a prevalence of thyroid dysfunction is higher in the older adults as compared to the younger population [1,2]. Advancing age is known to influence the formation of adenomatous goiter and thyroid cancer [3]. The prevalence of thyroid nodules is increased in the older adults, reaching a frequency of nearly 50% by the age of 65 [4]. Both prevalence and aggressiveness of thyroid cancer increase with age [2]. Women are affected by thyroid nodule and cancer two to five times more often than men, but in age over 65 years a prevalence of thyroid cancer may be higher in men [2-4, 5].

Aging is characterized by progressive impairment of body functions caused by the accumulation of molecular damage in DNA, proteins and lipids, is also characterized by an increase in intracellular oxidative stress due to the progressive decrease of the intracellular reactive oxygen species (ROS) scavenging [6,7]. Oxidative damage to cellular macromolecules which induce age-related diseases, including cancer, can also arise through overproduction of ROS and faulty antioxidant and/or DNA repair mechanisms [8]. Overproduction of ROS is associated with stress, inflammation, radiation, and some other factors, including overload of certain chemical elements, in both blood and certain tissues, or deficiency of other chemical elements with antioxidant properties [9-15]. The imbalance in the composition of chemical elements in cells, tissues and organs may cause different types of pathology. The importance of appropriate levels of many chemical elements is indisputable, due to their beneficial roles when present in specific concentration ranges, while on the other hand they can cause harmful effects with excessively high or low concentrations [12].

In our previous studies [16-24] the high mass fraction of iodine and some other chemical element were observed in intact human thyroid gland when compared with their levels in non-thyroid soft tissues of the human body. However, the age-dependence of chemical element mass fraction in thyroid of adult and, particularly, older males is still need to be evaluated. One valuable way to elucidate the situation is to compare the mass fractions of chemical elements in young adult (the control group) with those in older adult and geriatric thyroid. The other way is to calculate a correlation between age and chemical element content in thyroid. The findings of the excess or deficiency of chemical element contents in thyroid and the perturbations of their relative proportions in glands of adult and older males may indicate their roles in a higher prevalence of thyroid dysfunction in the older adults.

The reliable data on chemical element mass fractions in normal geriatric thyroid is apparently extremely limited. There are a few studies reporting chemical element content in human thyroid, using chemical techniques and instrumental methods [25-45]. However majority of the analytical
methods currently used and validated for the determination of major and trace elements in thyroid and other human organs are based on techniques requiring sample digestion. The most frequently used digestion procedures are the traditional dry ashing and high-pressure wet digestion that cause destruction of organic matter of the sample. Sample digestion is a critical step in elemental analysis and due to the risk of contamination and analytes loss, a digestion step contributes to the systematic uncontrolled analysis errors [46-48]. Moreover, only a few of the previous studies employed quality control using certified/standard reference materials (CRM/SRM) for determination of the chemical element mass fractions. Herein, in published papers there is no data on the age effect on chemical element contents in normal human thyroid.

Therefore, sample-nondestructive technique such as instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) combined in consecutive order with destructive inductively coupled plasma atomic emission spectrometry (ICP-AES) is good alternatives for multi-element determination in the samples of thyroid parenchyma. This combination provides a possibility of data quality assurance using a comparison of results obtained for some elements by both methods.

There were three aims in this study. The primary purpose of the study was to determine reliable values for chemical element mass fractions in the normal (intact) thyroid of subjects ranging from children to older males using INAA-SLR and ICP-AES. The second aim was to compare the chemical mass fractions determined in thyroid gland of age group 2 (adults and elderly persons aged 36 to 80 years), with those of group 1 (from 20 to 35 years). The final aim was to find the correlations between age and chemical element contents.

Materials and Methods

Ethics approval

The study plan was submitted to the Ethical Committee of the Medical Radiological Research Centre, Obninsk, Russia for approval prior to starting the study, as required by local regulatory provisions, and the Declaration of Helsinki.

Samples

Samples of the human thyroid were obtained from randomly selected autopsy specimens of 72 males (European-Caucasian) aged 2 to 80 years. All the deceased were citizens of Obninsk and had undergone routine autopsy at the Forensic Medicine Department of City Hospital, Obninsk. From all subjects were selected two age groups, group 1 with 20-35 years (26.0±1.0 years, M±SEM, n=27) and group 2 with 36-80 years (53.3±2.5 years, M±SEM, n=36). These groups were selected to reflect the condition of thyroid tissue in the young adults and first period of adult life (group 1) and in the second period of adult life as well as in old age (group 2). The available clinical data were reviewed for each subject. None of the subjects had a history of an intersex condition, endocrine disorder, or other chronic disease that could affect the normal development of the thyroid. None of the subjects were receiving medications or used any supplements known to affect thyroid chemical element contents. The typical causes of sudden death of most of these subjects included trauma or suicide and also acute illness (cardiac insufficiency, stroke, embolism of pulmonary artery, alcohol poisoning).

Sample preparation

All right lobes of thyroid glands were divided into two portions using a titanium scalpel [49]. One tissue portion was reviewed by an anatomical pathologist while the other was used for the chemical element content determination. A histological examination was used to control the age norm conformity as well as the unavailability of microadenomatosis and latent cancer.

After the samples intended for chemical element analysis were weighed, they were transferred to -20°C and stored until the day of transportation in the Medical Radiological Research Center, Obninsk, where all samples were freeze-dried and homogenized [50-52].

The sample weighing about 100 mg was used for chemical element measurement by INAA-SLR. The samples for INAA-SLR were sealed separately in thin polyethylene films washed beforehand with acetone and rectified alcohol. The sealed samples were placed in labeled polyethylene ampoules. Biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used as standards [53]. In addition to BSS, aliquots of commercially available pure compounds were also used.

After NAA-SLR investigation the thyroid samples were taken out from the polyethylene ampoules and used for ICP-AES. The samples were decomposed in autoclaves: 1.5 mL of concentrated HNO3 (nitric acid at 65 %, maximum (max) of 0.0000005 % Hg; Gr. ISO, Merck Darmstadt, Germany) and 0.3 mL of H2O2 (pure for analysis) were added to thyroid samples, placed in one-chamber autoclaves (Ancon-AT2, Ltd., Moscow, Russia) and then heated for 3 h at 160-200 °C. After autoclaving, they were cooled to room temperature and solutions from the decomposed samples were diluted with deionized water (upto 20 mL) and transferred to plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without tissue samples (only HNO3+H2O2+ deionized water), and the resultant solutions were used as control samples.

Certified Reference Materials

For quality control, ten subsamples of the certified reference materials IAEA H-4 Animal Muscle from the International Atomic Energy Agency (IAEA), and also five sub-samples INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2 Mixed Polish Herbs from the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) were analyzed simultaneously with the investigated thyroid tissue samples. All samples of CRM were treated in the same way as the thyroid tissue samples. Detailed results of this quality assurance program were presented in earlier publications [54-56].

Instrumentation and methods

A horizontal channel equipped with the pneumatic rabbit system of the WWR-C research nuclear reactor was applied to determine the mass fraction of Br, Ca, K, Mg, Mn, and Na by INAA-SLR. The neutron flux in the channel was 1.7×10^4 n cm-2 s-1. Ampoules with thyroid samples, BSS, intra laboratory-made standards, and CRM were put into polyethylene rabbits and then irradiated separately for 180s. Copper foils were used to assess neutron flux. The measurement of each sample was made twice, 1 and 120 min after irradiation. The duration of the first and second measurements was 10 and 20 min,
respectively. The gamma spectrometer included the 100 cm Ge detector and on-line computer-based MCA system. The spectrometer provided a resolution of 1.9 keV on the 1332 keV line.

Sample aliquots were used to determine the Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, Si, Sr, V, and Zn mass fractions by ICP-AES using the Spectrometer ICAP-61 (Thermo Jarrell Ash, USA). The determination of the trace element content in aqueous solutions was made by the quantitative method using calibration solutions (High Purity Standards, USA) of 0.5 and 10 mg/L of each element. The calculations of the trace element content in the probe were carried out using software of a spectrometer (ThermoSPEC, version 4.1).

Table 2: Some statistical parameters of Al, B, Ba, Br, Ca, Cu, Fe, I, K, Li, Mg, Mn, Na, P, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in thyroid of male (n=72).

| Element | Mean | SD | MIN | MAX | Median | P 0.025 | P 0.975 |
|---------|------|----|-----|-----|--------|---------|---------|
| Al      | 11.3 | 14.9 | 2.2 | 800 | 69.3   | 6.40    | 11.2    | 56.6     |
| B       | 0.491| 0.473| 0.071| 0.200| 2.300  | 0.300   | 0.200   | 2.03     |
| Ba      | 1.03 | 1.08 | 0.16 | 0.050| 4.70   | 0.570   | 0.141   | 4.08     |
| Br      | 13.7 | 7.8  | 1.0  | 1.90 | 32.3   | 10.2    | 2.50    | 30.7     |
| Ca      | 1675 | 979  | 122 | 373 | 5582   | 1458   | 429     | 4163     |
| Cl      | 3449 | 1450 | 219 | 1030| 5920   | 3470   | 1262    | 5657     |
| Cu      | 4.07 | 1.41 | 0.11 | 1.10 | 11.0   | 4.00    | 1.93    | 6.21     |
| Fe      | 223  | 95   | 12  | 592 | 489    | 215    | 77.5    | 445      |
| I       | 1786 | 940  | 118 | 220 | 4205   | 1742   | 239     | 3808     |
| K       | 6800 | 2352 | 299 | 1689| 5293   | 6060   | 3734    | 12355    |
| Li      | 0.0225| 0.0168| 0.0028| 0.0400| 0.0077 | 0.0179| 0.0046 | 0.0547 |
| Mg      | 316  | 136  | 18  | 99.0| 930    | 287    | 118     | 572      |
| Mn      | 1.27 | 0.47 | 0.06 | 0.470| 2.30   | 1.16    | 0.534   | 2.21     |
| Na      | 7094 | 1709 | 206 | 3700| 13453  | 6882   | 4003    | 11350    |
| P       | 4414 | 1366 | 204 | 2127| 8996   | 4227   | 2305    | 6858     |
| S       | 8745 | 1478 | 220 | 5066| 11377  | 8806   | 5925    | 11326    |
| Si      | 43.6 | 41.1 | 6.1 | 5.70| 180   | 32.7   | 7.45    | 163      |
| Sr      | 3.96 | 2.98 | 0.39 | 0.100| 12.6   | 2.95    | 0.443   | 11.7     |
| V       | 0.104| 0.033| 0.005| 0.0510| 0.200  | 0.100   | 0.0591 | 0.179    |
| Zn      | 95.4 | 39.8 | 5.1 | 34.0| 237    | 87.6   | 45.2    | 199      |

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.

Information detailing with the NAA-SLR and ICP-AES methods used and other details of the analysis was presented in our previous [54-58].

Table 3: Median, minimum and maximum value of means Al, B, Ba, Br, Ca, Cu, Fe, I, K, Li, Mg, Mn, Na, P, Si, Sr, V, and Zn 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)* | Minimum of means M or M±SD, (n)** | Maximum of means M or M±SD, (n)** |
| Al      | 33.6 (12)                | 0.33 (-) [25] | 420 (25) [26] | 11.3±14.9 |
| B       | 0.151 (2)                | 0.084 (3) [27] | 0.46 (3) [27] | 0.49±0.473 |
| Ba      | 0.67 (7)                 | 0.0084 (83) [28] | ≤5.0 (16) [29] | 1.03±1.08 |
| Br      | 18.1 (11)                | 5.12 (44) [30] | 284±44 (14) [31] | 13.7±7.8 |
| Ca      | 1600 (17)                | 840±240 (10) [32] | 3800±320 (29) [32] | 1675±979 |
| Cl      | 6800 (5)                 | 804±80 (4) [33] | 8000 (4) [34] | 3449±1450 |
| Cu      | 6.1 (57)                 | 1.42 (120) [35] | 220±22 (10) [33] | 4.07±1.41 |
| Fe      | 252 (21)                 | 56 (120) [35] | 244±70 (14) [31] | 223±95 |
| I       | 1888 (95)                | 159±8 (23) [36] | 5772±2708 (50) [37] | 1786±940 |
| K       | 4400 (17)                | 46.4±4.8 (4) [33] | 6090 (17) [29] | 668±2352 |
| Li      | 6.3 (2)                  | 0.092 (-) [38] | 12.6 (180) [39] | 0.0225±0.0168 |
| Mg      | 390 (16)                 | 3.5 (-) [25] | 840±400 (14) [40] | 316±136 |
| Mn      | 1.82 (36)                | 0.44±11 (12) [41] | 69.2±7.2 (4) [42] | 1.27±0.47 |
| Na      | 8000 (9)                 | 438 (-) [42] | 10000±5000 (11) [40] | 7094±1709 |
| P       | 3200 (12)                | 16 (7) [43] | 7520 (60) [30] | 4414±1366 |
| S       | 11000 (3)                | 4000 (-) [34] | 11800 (44) [30] | 8745±1578 |
| Si      | 16.0 (3)                 | 0.97 (-) [25] | 143±60 (40) [44] | 43.6±4.11 |
| Sr      | 0.73 (9)                 | 0.55±0.26 (21) [27] | 46.8±4.6 (4) [33] | 3.96±2.98 |
| V       | 0.042 (6)                | 0.012 (2) [45] | 18±2 (4) [33] | 0.104±0.033 |
| Zn      | 118 (51)                 | 32 (120) [35] | 820±204 (14) [31] | 95.4±39.8 |

M - arithmetic mean, SD - standard deviation, (n) - number of all references, (n)** - number of samples.

Citation: Zaichkiv V and Zaichkiv S. Association between Age and Twenty Chemical Element Contents in Intact Thyroid of Males. SM Gerontol Geriatr Res. 2018; 2(1): 1014.

https://dx.doi.org/10.36876/smggr.1014
Results

The comparison of our results for the Ca, K, Mg, Mn, and Na mass fractions (mg/kg, dry mass basis) in the normal thyroid of male obtained by both NAA-SLR and ICP-AES methods is shown in Table 1.

Table 2 represents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, percentiles with 0.025 and 0.975 levels) of the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, Si, Sr, V, and Zn mass fractions in intact (normal) thyroid of males.

The comparison of our results with published data for the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, Si, Sr, V, and Zn contents in the human thyroid is shown in Table 3.

Table 4 indicates some statistical parameters of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in intact male thyroid of two age groups 20-35 and 36-80 years.

### Table 4: Some statistical parameters of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in male thyroid of two age groups 20-35 and 36-80 years.

| Age          | Element | M   | SD  | SEM  | Min | Max  | Median | P 0.025 | P 0.975 |
|--------------|---------|-----|-----|------|-----|------|--------|---------|---------|
| 20-35 years  | Al      | 12.3 | 16.6 | 3.7  | 1.10 | 60.3 | 4.50   | 1.20    | 52.0    |
| n=27         | B       | 0.630 | 0.622 | 0.139 | 0.020 | 2.300 | 0.340  | 0.200   | 2.21    |
| n=27         | Br      | 12.0 | 8.3  | 1.9  | 1.90 | 32.3 | 10.0   | 2.19    | 28.1    |
|              | Ca      | 1567 | 528  | 104  | 414 | 2880 | 1484  | 599     | 2457    |
|              | Cl      | 3135 | 1489 | 413  | 1260 | 5660 | 2230  | 1359    | 5462    |
|              | Cu      | 3.80 | 0.91 | 0.19 | 2.30 | 5.60 | 3.90   | 2.35    | 5.44    |
|              | Fe      | 212  | 114  | 23   | 77.0 | 489  | 169   | 81.8    | 482     |
|              | I       | 1703 | 899  | 192  | 220 | 4205 | 1743  | 220     | 3526    |
|              | K       | 7073 | 1505 | 307  | 4490 | 10509 | 6812  | 4980    | 9854    |
|              | Li      | 0.0289 | 0.0221 | 0.021 | 0.0106 | 0.0977 | 0.0215  | 0.0119 | 0.0810 |
|              | Mg      | 327  | 100  | 21   | 168 | 522  | 294    | 169     | 509     |
|              | Mn      | 1.43 | 0.45 | 0.10 | 0.870 | 2.20 | 1.35 | 0.886 | 2.18 |
|              | Na      | 7246 | 1393 | 268  | 3700 | 10900 | 7216 | 4787 | 9819 |
|              | P       | 4500 | 1368 | 306  | 2127 | 6876 | 4167 | 2371 | 6769 |
|              | S       | 9329 | 1304 | 292  | 7507 | 11377 | 9217 | 7510 | 11370 |
|              | Si      | 44.5 | 38.2 | 8.5  | 5.70 | 168  | 36.3 | 7.36 | 133 |
|              | Sr      | 3.39 | 2.33 | 0.47 | 0.500 | 10.8 | 2.80 | 0.713 | 9.54 |
|              | V       | 0.106 | 0.038 | 0.009 | 0.0510 | 0.200 | 0.100 | 0.0596 | 0.191 |
|              | Zn      | 82.2 | 22.8 | 4.6  | 47.1 | 140  | 80.2 | 47.3 | 132 |
| 36-80 years  | Al      | 7.50 | 6.74 | 1.51 | 0.800 | 25.4 | 6.50 | 1.13 | 24.1 |
| n=36         | B       | 0.286 | 0.112 | 0.028 | 0.200 | 0.600 | 0.200 | 0.200 | 0.510 |
| n=36         | Br      | 15.0 | 7.5  | 1.3  | 4.50 | 31.1 | 13.6 | 4.58 | 30.4 |
|              | Ca      | 1860 | 1218 | 212  | 440 | 5582 | 1461 | 528 | 4796 |
|              | Cl      | 3478 | 1418 | 273  | 1030 | 5620 | 3500 | 1199 | 5594 |
|              | Cu      | 4.10 | 1.09 | 0.205 | 1.70 | 6.40 | 4.05 | 2.24 | 6.06 |
|              | Fe      | 224  | 79   | 15   | 52.0 | 389  | 233 | 69.6 | 367 |
|              | I       | 1930 | 970  | 169  | 254 | 4098 | 1994 | 262 | 3676 |
|              | K       | 6030 | 2379 | 414  | 3698 | 13300 | 5444 | 3724 | 11860 |
|              | Li      | 0.0150 | 0.0078 | 0.0019 | 0.0040 | 0.0335 | 0.0164 | 0.0043 | 0.0298 |
|              | Mg      | 296  | 165  | 31   | 99.0 | 930  | 281  | 104 | 707 |
|              | Mn      | 1.10 | 0.38 | 0.07 | 0.530 | 2.21 | 1.07 | 0.537 | 1.87 |
|              | Na      | 6872 | 1604 | 267  | 3930 | 12400 | 6719 | 4335 | 9970 |
|              | P       | 3964 | 909  | 203  | 2272 | 5676 | 4030 | 2428 | 5537 |
|              | S       | 8489 | 1479 | 331  | 5066 | 11000 | 8526 | 5470 | 10857 |
|              | Si      | 34.1 | 43.0 | 9.6  | 7.40 | 180  | 20.8 | 7.64 | 150 |
|              | Sr      | 4.58 | 3.52 | 0.68 | 0.100 | 12.6 | 3.70 | 0.750 | 12.3 |
|              | V       | 0.100 | 0.032 | 0.007 | 0.0590 | 0.170 | 0.095 | 0.0595 | 0.165 |
|              | Zn      | 95.2 | 33.6 | 6.4  | 34.0 | 176  | 87.6 | 44.3 | 167 |

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.

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https://dx.doi.org/10.36876/smggr.1014
Table 5: Differences between mean values (M±SEM) of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, Li, Mg, Mn, Na, P, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in normal male thyroid of two age groups (AG1 and AG2).

| Element | AG1 20-35 years | AG2 36-80 years | t-test | U-test | AG2 to AG1 |
|---------|-----------------|-----------------|--------|--------|------------|
| Al      | 12.3±16.6       | 7.50±1.51       | 0.243  | >0.05  | 0.61       |
| B       | 0.630±0.622     | 0.286±0.026     | 0.024  | <0.001 | 0.45       |
| Ba      | 1.21±1.08       | 0.568±0.079     | 0.023  | <0.001 | 0.47       |
| Br      | 12.0±8.3        | 15.0±1.3        | 0.190  | >0.05  | 1.25       |
| Ca      | 1567±528        | 1880±212        | 0.192  | >0.05  | 1.20       |
| Cl      | 3135±1489       | 3478±273        | 0.494  | >0.05  | 1.11       |
| Cu      | 3.80±0.91       | 4.10±0.21       | 0.293  | >0.05  | 1.08       |
| Fe      | 212±114         | 224±15          | 0.656  | >0.05  | 1.06       |
| I       | 1703±899        | 1930±169        | 0.378  | >0.05  | 1.13       |
| K       | 7073±1505       | 6030±414        | 0.048  | <0.001 | 0.85       |
| Li      | 0.0289±0.00221  | 0.0150±0.0019   | 0.033  | <0.001 | 0.52       |
| Mg      | 327±100         | 296±31          | 0.419  | >0.05  | 0.91       |
| Mn      | 1.43±0.45       | 1.10±0.07       | 0.009  | <0.001 | 0.79       |
| Na      | 7246±1393       | 6872±267        | 0.327  | >0.05  | 0.95       |
| P       | 4500±1368       | 3964±203        | 0.154  | >0.05  | 0.88       |
| S       | 9329±1304       | 8489±331        | 0.064  | >0.05  | 0.91       |
| Si      | 44.5±38.2       | 34.1±6.9        | 0.424  | >0.05  | 0.77       |
| Sr      | 3.39±2.33       | 4.58±0.68       | 0.158  | >0.05  | 1.35       |
| V       | 0.106±0.038     | 0.100±0.007     | 0.057  | >0.05  | 0.94       |
| Zn      | 82.2±22.8       | 95.2±6.4        | 0.053  | >0.05  | 1.16       |

M – Arithmetic mean, SEM – standard error of mean, t-test - Student’s t-test, U-test - Wilcoxon-Mann-Whitney U-test, statistically significant values are in bold.

To estimate the effect of age on the chemical element contents we examined two age groups, described above (Table 5). In addition, the Pearson correlation coefficient between age and chemical element mass fraction was calculated (Table 6). Figure 1 shows the individual data sets for the integral Al, B, Ba, Ca, I, K, Li, Mn, and P mass fraction in all samples of thyroid, and also lines of trend with age. Since the age dependency of these element contents was best described by a polynomial function, this approximation was reflected in Figure 1.

Discussion

Precision and accuracy of results

A good agreement of our results for the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, Si, Sr, V, and Zn mass fractions with the certified values of CRM IAEA H-4 Animal Muscle, INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2Mixed Polish Herbs[54-56] as well as the similarity of the means of the Ca, K, Mg, Mn, and Na mass fractions in the normal thyroid of male determined by both NAA-SLR and ICP-AES methods (Table 1) demonstrates an acceptable precision and accuracy of the results obtained in the study and presented in Tables 2-6 and Figure 1.

Comparison with published data

The obtained means for Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Mg, Mn, Na, P, Si, Sr, V, and Zn mass fraction, as shown in Table 3, agree well with the medians of mean values reported by other researches for the human thyroid, including samples received from persons who died from different non-thyroid diseases [25-45]. The obtained mean for Li is two orders of magnitude lower the median of previously reported data. Moreover, it is outside the range of previously reported means. A number of values for chemical element mass fractions were not expressed on a dry mass basis by the authors of the cited references. Hence we calculated these values using published data for water 75% [60] and ash 4.16% on dry mass basis [61] contents in thyroid of adults.

Effect of age on chemical element contents

A statistically significant age-related decrease in B, Ba, K, Li, and Mn mass fraction was observed in thyroid of males when two age groups were compared (Table 5). In second group of males with mean age 53.3 years the mean of B, Ba, K, Li, and Mn mass fraction in thyroids were 55%, 53%, 15%, 48%, and 21%, respectively, lower than in thyroids of the first age group (mean age 26.0 years). A statistically significant decrease in B, Ba, K, Li, and Mn mass fraction with age was confirmed by the Pearson’s coefficient of correlation between age and mass fractions of these elements (Table 6). Moreover, a statistically significant increase in Ca and I content accompanied by a decrease in Al and P was shown by the Pearson’s coefficient of correlation between age and mass fractions of these elements (Table 6, Figure 1).

Thus, both positive (increase with age) and negative (decrease with age) correlations were observed for chemical element contents in the normal male thyroid. The Al, B, Ba, K, Li, Mn, and P contents (Figure 1) were declining with age. The rate of the decrease was clearly larger for Al, B, and Ba than for K, Li, Mn, and P (Figure 1). The decrease of Al, B, Ba, K, Li, and P content was fast during the first four-five decades, after which the changes in mass fractions of these elements were rather limited. However, the Mn mass fraction showed a progressive slow decrease with age in range from 2 to 80 years. Ca and I, in contrast, are accumulated during lifespan (Figure 1). Herein, elemental contents remain fairly stable until the fifth decade, after which a strong but variable accumulation takes place. Ca and I mass fractions are about doubled from age 40 until age 80(Figure 1).

As per author’s current information, no published data referring to age-related changes of Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, Si, Sr, V, and Zn mass fractions in human thyroid is available.

Table 6: Correlations between age (years) and chemical element mass fractions (mg/kg, dry mass basis) in the normal thyroid of male (r - coefficient of correlation).

| Element | Al   | B    | Ba   | Br   | Ca   | Cl   | Cu   | Fe   | I    | K    |
|---------|------|------|------|------|------|------|------|------|------|------|
| r       | -0.29*| -0.37*| -0.41*| 0.11 | 0.45*| 0.11 | -0.09 | -0.11 | 0.32*| -0.32*|
| r       | -0.31*| 0.08  | -0.35*| -0.10| -0.30*| -0.14| -0.24| 0.09  | -0.02| -0.10|

Statistically significant values: *p<0.05, **p<0.01, ***p<0.001.

Citation: Zaichick V and Zaichick S. Association between Age and Twenty Chemical Element Contents in Intact Thyroid of Males. SM Gerontol Geriatr Res. 2018; 2(1): 1014.
https://dx.doi.org/10.36876/smggr.1014
Role of chemical elements in malignant transformation of the thyroid

**Calcium:** Ca content increases with age in many organs of human body [62-70]. This natural phenomenon is probably a common occurrence for all organs and soft tissues, except for bone. An age-related increase and excess in Ca mass fractions in thyroid tissue may contribute to harmful effects on the gland. There are good reasons for such speculations since many reviews and numerous papers raise the concern about role of Ca in the prostate, breast, lung and other organ malignant transformation [62-72]. Calcium ions Ca$^{2+}$ are central to both cell proliferation and cell death [64]. Changes in cytosolic Ca$^{2+}$ trigger events critical for tumorigenesis, such as cellular motility, proliferation and apoptosis [66]. An increased growth rate of cells is correlated with an increase in the intracellular calcium pool content [62,63]. Moreover, increases in cytosolic free Ca$^{2+}$ represent a ubiquitous signaling mechanism that controls a variety of cellular processes, including not only proliferation, but also cell metabolism and gene transcription [65]. Indeed, an increased level of Ca content in the thyroid tissue of old males reflects an increase in the intracellular calcium pool. Thus, an increase of Ca content in tissue and organs with age is a key feature in etiology of many benign and malignant tumors, including thyroid goiter and cancer.

**Iodine:** The endocrine organs, including the thyroid gland, undergo important functional changes during aging. The prevalence of subclinical hypothyroidism, which is characterized by normal free thyroxine and increased thyrotropin (TSH) levels, increases with aging. Therefore, subclinical hypothyroidism is frequently seen in old people [73]. In this, probably, the intra-thyroidal iodine levels are elevated in old men with increased TSH level. However, it is well known that excess in I mass fractions in thyroid tissue may contribute to harmful effects on the gland, including malignant transformation [19-21, 74-77].
Aluminum: Trace element Al is not described as essential, because no biochemical function has been directly connected to it. At this stage of our knowledge, no doubt that the Al overload negatively impacts human health [78]. Why Al content in normal male thyroid decrease with age and how it acts on thyroid are still to be cleared.

Boron: Trace element B is known to influence the activity of many enzymes [79]. Numerous studies have demonstrated beneficial effects of B on human health, including anti-inflammatory stimulus - reduces levels of inflammatory biomarkers, such as high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor α (TNF-α); as well as raises levels of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase [80]. Moreover, B helps the conversion of the storage form of thyroid hormone, T4, to T3, the active form. In normal situations B deficiency does not occur in humans, as most of the dietary sources are rich in this nutrient, but older adults are at greater risk for nutritional deficiencies than are younger adults. Thus, the age-related decrease of B content in normal male thyroid can be associated with a decrease in B dietary intake.

Barium: Trace element Ba has been shown to affect thyroid function in occupationally exposed persons. Urinary Ba content was associated with decreased T4 and T3 level in blood serum [81]. Why Ba content in normal male thyroid decrease with age are still to be cleared.

Potassium: K is a ubiquitous, intracellular element essential to more than one metabolic pathway. Low K levels in the blood and tissue, known as hypokalemia, is particularly common in the elderly for various age-related reasons. A deficiency in potassium is not immediately evident and develops gradually over time in most people [82]. Thus, the age-related decrease of K content in normal male thyroid reflects the process of hypokalemia development in senior citizens. From the other hand, because K+ is mainly an intracellular electrolyte, a decreased level of K content in the thyroid tissue of old males might indicate an age-related decrease of ratio “thyroid cell mass - follicular colloid mass”. Herein, mass fraction of Na does not change during a lifespan (Table 5). From this it follows that an intracellular Na+: K+ ratio in thyroid of old males may be higher normal level. In turn, increasing intracellular Na+: K+ ratio is associated with a depolarization of the cell membrane [83]. The sustained depolarization of the cell membrane results in an increased rate of cell division and in that way with an increased risk of goiter, benign and malignant tumor of thyroid.

Lithium: Li has been used for decades to treat bipolar disorders. Impaired thyroid function is a common side effect of Li medication. Recent data indicate that Li exposure through drinking water, although providing much lower doses than the medication, may also affect thyroid hormone levels. The inhibition of thyroid hormone secretion results in decreased serum T4 and T3 concentrations [84]. From the other hand, the results of lifelong lithium-poor nutrition of animals show that lithium is essential to the fauna, and thus, to humans as well. It was shown that lithium-poor nutrition has a negative influence on feed intake, organism growth, skin properties, reproduction performance, milk production, mortality, and on some enzyme activity, mainly the enzymes of the citrate cycle, glycolysis, and of nitrogen metabolism [85]. Thus, Li is an ambivalent element for which further studies are necessary to determine the likely narrow range of content in thyroid with beneficial effects.

The Li intake by adult humans with mixed diets was systematically investigated in 10 test populations from different regions of Germany [86]. Adults of both genders with mixed diets consumed significantly more Li with increasing age. On average, seniors consumed 30-46% more Li. If Li dietary intake by Russian population is similar to the situation in Germany, mechanisms of the age-related decrease of Li content in normal male thyroid can’t be completely elucidated. In our previous study significant age-related decrease in Li content in prostate gland was observed [85].

Manganese: Trace element Mn is a cofactor for numerous enzymes, playing many functional roles in living organisms. The Mn-containing enzyme, manganese superoxide dismutase (Mn-SOD), is the principal antioxidant enzyme which neutralizes the toxic effects of reactive oxygen species. It has been speculated that Mn interferes with thyroid hormone binding, transport, and activity at the tissue level [87]. There is opinion that Mn deficiencies in humans are rare and humans maintain stable tissue levels of this trace element [88]. This contradicts the observed age-related decrease of Mn content in normal male thyroid parenchyma.

It was reported that intracellular Mn content was positively correlated with manganese-containing superoxide dismutase (Mn-SOD), suggesting that the intracellular Mn level is associated with Mn-SOD activity [89]. Thus, a decrease of Mn content in thyroid parenchyma with age indicates the deficiency of antioxidant enzymes in the gland of old males.

A number of nutritional deficiencies are known to develop in subclinical hypothyroidism, including mineral deficiency: P, Mn, K and others [90]. At the current state of knowledge, it is impossible to reach definite conclusion of the cause- and-effect relation between subclinical hypothyroidism and deficiency of P, Mn, K as well as some others chemical elements in old male thyroid. However, if the intra-thyroidal contents of chemical element in the first period of male life (<36 years) accept as normal levels, we can conclude that in the thyroid of old men there is at least deficiency of P, Mn, and K.

Study Limitations

This study has two limitations. Firstly, analytical techniques employed in this study measure only twenty trace element (Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of chemical elements investigated in thyroid tissue. Secondly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on tissue Al, B, Ba, Ca, I, K, Li, Mn, and P level alteration with age and shows the necessity the need to continue age-dependence research of chemical element contents in male thyroid.

Conclusion

Our data elucidate that there is a statistically significant increase in Ca and I mass fraction, as well as a decrease in Al, B, Ba, K, Li, Mn, and P mass fraction in the normal thyroid of male during a lifespan. Thus, from results of our study, a goitrogenic and carcinogenic effect of inadequate Al, B, Ba, Ca, I, K, Li, Mn, and P levels in the thyroids of older males is shown to be a very likely consequence.
Acknowledgements

We are grateful to Dr. Yu Choporov, Head of the Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples. We are also grateful to Dr. Karandas chev V, Dr. Nosenko S, and Moskvina I, Institute of Microelectronics Technology and High Purity Materials, Chernogolovka, Russia, for their help in ICP-MS analysis.

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Citation: Zaichick V and Zaichick S. Association between Age and Twenty Chemical Element Contents in Intact Thyroid of Males. SM Gerontol Geriatr Res. 2018; 2(1): 1014. https://dx.doi.org/10.36876/smgergr.1014
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