ISSN: 2833-4353

Research Article

Archives of Epidemiology & Public Health Research

Relationships Between Iodine and Some Trace Elements in Normal Thyroid of Males Investigated by Neutron Activation Analysis

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Submitted: 05 Jan 2023; **Accepted**: 15 Jan 2023: **Published**: 27 Jan 2023

Citation: *Vladimir Zaichick (2023). Relationships Between Iodine and Some Trace Elements in Normal Thyroid of Males Investigated by Neutron Activation Analysis. Arch Epidemiol Pub Health Res, 2(1), 154-160.*

Abstract
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 contents in thyroid play important role in goitro- and carcinogenesis of gland. The correlations with age of the ten trace element (TE) contents (Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn), I/Ag, I/Co, I/Cr, I/Fe, I/Hg, I/Rb, I/Sb, I/Sc, I/Se, and I/Zn content ratios, and inter relationships between TE contents and I/TE content ratios in normal thyroid of 73 males (mean age 37.3 years, range 2.0-80) was investigated by neutron activation analysis. Our data reveal that the I and Se content, as well as the I/Cr and I/Rb content ratio increase in the normal thyroid of male during a lifespan. Therefore, a goitrogenic and tumorogenic effect of excessive I and Se level in the thyroid of old males and of disturbance in intrathyroidal I/Cr and I/Rb relationships with increasing age may be assumed. Furthermore, it was found that the levels of Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn in the thyroid gland are interconnected and depend on the content of I in it. Because I plays a decisive role in the function of the thyroid gland, the data obtained allow us to conclude that, along with I, such TEs as Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn, if not directly, then indirectly, are involved in the process of thyroid hormone synthesis.

Keywords: Thyroid; Trace Elements; Age-Related Changes; Intrathyroidal Trace Elements Relationships; Neutron Activation Analysis

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 [25-39]. 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 [40-52]. 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 silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), iodine (I), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), and zinc (Zn) mass fractions in the normal thyroid of subjects ranging from children to elderly males using instrumental neutron activation analysis (INAA) and calculate individual values of I/Ag, I/Co, I/Cr, I/Fe, I/Hg, I/Rb, I/Sb, I/Sc, I/Se, and I/Zn . The second aim was to compare the Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn mass fractions in thyroid gland obtained in the study with published data. The final aim was to estimate the inter-correlations of TE contents and I/TE content ratios in normal thyroid of males and changes of these parameters with age.

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

Samples of the human thyroid were obtained from randomly selected autopsy specimens of 73 males (European-Caucasian) aged 2.0 to 80 years. All the deceased were citizens of Obninsk and had undergone routine autopsy at the Forensic Medicine Department of City Hospital, Obninsk. 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 TE 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). All right lobes of thyroid glands were divided into two portions using a titanium scalpel [53]. One tissue portion was reviewed by an anatomical pathologist while the other was used for the TE 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 TE 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 [54]. To determine the contents of the TE by comparison with a known standard, aliquots of commercial, chemically pure compounds were used [55]. Ten subsamples of the Certified Reference Material (CRM) IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) were analyzed to estimate the precision and accuracy of results. The CRM IAEA H-4 and IAEA HH-1 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 1.7×10^{13}n cm^{-2} s^{-1}. A vertical channel of nuclear reactor WWR-c with a neutron flux of 1.3□10^{13} 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 [56-57].

A dedicated computer program for INAA-SLR mode optimization was used [58]. All thyroid samples were prepared in duplicate, and mean values of TE contents were used in final calculation. Using Microsoft Office Excel, a summary of the statistics, including, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for TE contents and I/TE content ratios. Pearson's correlation coefficient was used in Microsoft Office Excel to calculate the relationship "age – TE mass fraction", as well as to identify inter-thyroidal relationships between different TE contents and between different TE content ratios.

Results

Table 1 depicts comparison of our data for ten TE in ten sub-samples of CRM IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) with the corresponding certified values of TE contents in these materials.

Table 1. INAA-LLR data of trace element contents in certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) compared to certified values ((mg/kg, dry mass basis)

Element	IAEA H-4 animal muscle	This work results	IAEA HH-1 human hair	This work results
	95% confidence interval	M±SD	95% confidence interval	M±SD
Ag	-	0.033±0.008	0.19[b]	0.18±0.05
Co	0.0027[b]	0.0034±0.0008	5.97±0.42[a]	5.4±1.1
Cr	0.06[b]	0.071±0.010	0.27[b]	≤0.3
Fe	49.1±6.5[a]	47.0±1.0	23.7±3.1[a]	25.1±4.3
Hg	0.014[b]	0.015±0.004	1.70±0.09[a]	1.54±0.14
I	0.08±0.10[b]	<1.0	20.3±8.9[b]	19.1±6.2
Rb	18.7±3.5[a]	23.7±3.7	0.94[b]	0.89±0.17
Sb	0.0056[b]	0.0061±0.0021	0.031[b]	0.033±0.009
Sc	0.0059[b]	0.0015±0.0009	-	-
Se	0.28±0.08[a]	0.281±0.014	0.35±0.02[a]	0.37±0.08
Zn	86.3±11.5[a]	91±2	174±9[a]	173±17
M – arithmetical mean, SD – standard deviation, a – certified values, b – information values.				

Table 2 represents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn mass fractions, as well as I/Ag, I/Co, I/Cr, I/Fe, I/Hg, I/Rb, I/Sb, I/Sc, I/Se, and I/Zn mass fraction ratios in normal thyroid of males.

Table 2. Some statistical parameters of Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry tissue) as well as I/Ag, I/Co, I/Cr, I/Fe, I/Hg, I/Rb, I/Sb, I/Sc, I/Se, and I/Zn mass fraction ratios in normal male thyroid (n=73)

	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Ag	0.0156	0.0155	0.0021	0.0017	0.0800	0.0104	0.0018	0.0661
Co	0.0352	0.0234	0.0031	0.0046	0.124	0.0302	0.0113	0.101
Cr	0.520	0.286	0.041	0.130	1.30	0.414	0.152	0.980
Fe	222	96	12	51.0	487	221	76.1	432
Hg	0.0461	0.0391	0.0053	0.0091	0.180	0.0324	0.0102	0.150
I	1486	902	130	220	3744	1337	222	3443
Rb	7.89	4.56	0.58	2.24	29.4	6.86	2.73	18.2
Sb	0.108	0.076	0.010	0.0047	0.308	0.0965	0.0095	0.291
Sc	0.0051	0.0036	0.0012	0.0005	0.0118	0.0044	0.0007	0.0112
Se	2.36	1.34	0.17	0.530	5.80	1.96	0.804	5.70
Zn	103	43	5.5	34.0	221	94.6	40.5	200
I/Ag	231906	258404	38100	6430	1372273	147971	6614	984706
I/Co	61704	39524	5646	4911	153211	54981	7086	142590
I/Cr	4063	3715	580	358	17005	2799	373	15817
I/Fe	10.5	9.8	1.3	0.800	57.9	7.62	1.05	31.2
I/Hg	66473	56184	8375	2000	267834	46377	4220	168940
I/Rb	305	275	38	13.5	1271	225	20.5	1120
I/Sb	24381	26094	3654	1803	132553	14859	2283	93804
I/Sc	750246	541736	191533	106271	1588000	807159	122714	1526534
I/Se	917	754	106	83.0	3708	746	134	3095
I/Zn	20.8	13.8	1.9	1.64	66.9	21.2	2.07	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.								

The comparison of our results with published data for the Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn contents in the human thyroid is shown in Table 3.

Table 3. Median, minimum and maximum value of means Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in normal human thyroid according to data from the literature in comparison with our results (mg/kg, dry tissue)

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)**	Males and females M±SD
Ag	0.25 (12)	0.000784 (16) [40]	1.20±1.24 (105) [41]	0.0156±0.0155
Co	0.336 (17)	0.026±0.031 (46) [42]	70.4±40.8 (14) [43]	0.0352±0.0234
Cr	0.69 (17)	0.105 (18) [44]	24.8±2.4 (4) [45]	0.520±0.286
Fe	252 (21)	56 (120) [46]	2444±700 (14) [43]	222±96
Hg	0.08 (13)	0.0008±0.0002 (10) [47]	396±40 (4) [45]	0.0461±0.0391
I	1888 (95)	159±8 (23) [48]	5772±2708 (50) [49]	1486±902
Rb	12.3 (9)	≤0.85 (29) [47]	294±191 (14) [43]	7.89±4.56
Sb	0.105 (10)	0.040±0.003 (-) [50]	4.0 (-) [51]	0.108±0.076
Sc	0.009 (4)	0.0018±0.0003 (17) [52]	0.0135±0.0045 (10) [47]	0.0051±0.0036
Se	2.61 (17)	0.95±0.08 (29) [47]	756±680 (14) [43]	2.36±1.34
Zn	118 (51)	32 (120) [46]	820±204 (14) [43]	103±43

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

To estimate the effect of age on the TE contents and I/TE content ratios in normal thyroid of males Pearson's correlation coefficient was used (Table 4).

Table 4. Correlations between age (years) and trace element content (mg/kg, dry tissue), as well as between age and I/trace element mass fraction ratios in the normal male thyroid (r – coefficient of correlation)

El	Ag	Co	Cr	Fe	Hg	I	Rb	Sb	Sc	Se	Zn
r	-0.12	-0.12	-0.07	-0.05	0.20	0.32b	-0.06	0.02	-0.07	0.43c	0.06
Ratio	I/Ag	I/Co	I/Cr	I/Fe	I/Hg	I/Rb	I/Sb	I/Sc	I/Se	I/Zn	-
r	-0.08	0.13	0.34^{a}	0.23	0.01	0.28^{a}	0.06	0.14	-0.19	0.16	-

El - element, Statistically significant values: ^{a} p≤0.05, ^{b} p≤0.01, ^{c} p≤0.001.

The data of inter-thyroidal correlation (values of r – Pearson's coefficient of correlation) including all TE and I/TE content ratios identified by us are presented in Tables 5 and 6, respectively.

Table 5. Intercorrelations of the chemical element mass fractions in normal male thyroid (r – coefficient of correlation)

El	Co	Cr	Fe	Hg	I	Rb	Sb	Sc	Se	Zn
Ag	0.54^{c}	0.36^{a}	0.07	-0.13	-0.10	0.26	0.04	0.38	-0.06	-0.02
Co	1.00	0.39^{a}	0.06	-0.31^{a}	0.04	0.26	0.21	0.60^{a}	0.11	0.11
Cr	0.39^{a}	1.00	0.24	-0.33^{a}	-0.16	0.45^{b}	0.18	0.32	0.05	0.12
Fe	0.06	0.24	1.00	0.08	-0.28^{a}	-0.11	0.06	0.01	-0.01	0.22
Hg	-0.31^{a}	-0.33^{a}	0.08	1.00	-0.07	0.04	-0.13	-0.66^{a}	-0.03	0.13
I	0.04	-0.16	-0.28^{a}	-0.07	1.00	-0.26	0.31a	-0.51	0.53^{c}	-0.08
Rb	0.26	0.45^{b}	-0.11	0.04	-0.26	1.00	-0.11	0.80^{b}	-0.26	0.34^{a}
Sb	0.21	0.18	0.06	-0.13	0.31^{a}	-0.11	1.00	0.18	0.51^{c}	-0.20
Sc	0.60^{a}	0.32	0.01	-0.66^{a}	-0.51	0.80^{b}	0.18	1.00	0.30	-0.10
Se	0.11	0.05	-0.01	-0.03	0.53^{c}	-0.26	0.51^{c}	0.30	1.00	0.12
Zn	0.11	0.12	0.22	0.13	-0.08	0.32^{a}	-0.20	-0.10	0.12	1.00

El – element, Significant values: ^{a} p≤0.05, ^{b} p≤0.01, ^{c} p≤0.001.

Table 6. Intercorrelations of the I/trace element mass fraction ratios in normal male thyroid (r – coefficient of correlation)

Ratio	I/Co	I/Cr	I/Fe	I/Hg	I/Rb	I/Sb	I/Sc	I/Se	I/Zn
I/Ag	0.42[b]	0.45[b]	0.13	0.28	0.53[c]	0.28	-0.47	0.06	0.20
I/Co	1.00	0.52[c]	0.48[c]	0.22	0.59[c]	0.30[a]	0.37	0.37[b]	0.62[c]
I/Cr	0.52[c]	1.00	0.44[b]	0.21	0.69[c]	0.38[a]	0.17	0.37[a]	0.39[b]
I/Fe	0.48[c]	0.44[b]	1.00	0.47[c]	0.52[c]	0.12	0.28	0.23	0.50[c]
I/Hg	0.22	0.21	0.47[c]	1.00	0.45[b]	-0.13	-0.13	0.23	0.62[c]
I/Rb	0.59[c]	0.69[c]	0.52[c]	0.45[b]	1.00	0.11	0.40	0.15	0.65[c]
I/Sb	0.30[a]	0.38[a]	0.12	-0.13	0.11	1.00	0.32	0.46[c]	0.05
I/Sc	0.37	0.17	0.28	-0.13	0.40[a]	0.32	1.00	0.34	0.12
I/Se	0.37[b]	0.37[a]	0.23	0.23	0.15	0.46[c]	0.34	1.00	0.45[c]
I/Zn	0.62[c]	0.39[b]	0.50[c]	0.62[c]	0.65[c]	0.05	0.12	0.45[c]	1.00

Statistically significant values: [a] $p \leq 0.05$, [b] $p \leq 0.01$, [c] $p \leq 0.001$

Discussion

Good agreement of the Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn contents analyzed by INAA with the certified data of CRMs IAEA H-4 and IAEA HH-1 (Table 1) indicates an acceptable accuracy of the results obtained in the study for TE contents and I/TE content ratios in the normal male thyroid 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, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn mass fractions, as well as I/Ag, I/Co, I/Cr, I/Fe, I/Hg, I/Rb, I/Sb, I/Sc, I/Se, and I/Zn mass fraction ratios in normal thyroid of males (Table 2). 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 Med, 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.

Values obtained for Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in the normal human thyroid (Table 3) agree well with median of mean values reported by other researches [40-52]. The obtained means for Ag and Co were almost one order of magnitude lower median of previously reported means but inside the range of means (Table 3). Data cited in Table 3 also includes samples obtained from patients who died from different non-endocrine diseases. A number of values for TE mass fractions were not expressed on a dry mass basis by the authors of the cited references. However, we calculated these values using published data for water (75%) [42] and ash (4.16% on dry mass basis) [59] contents in thyroid of adults. No published data referring to I/Ag, I/Co, I/Cr, I/Fe, I/Hg, I/Rb, I/Sb, I/Sc, I/Se, and I/Zn mass fraction ratios in human thyroid was found.

With age, the I and Se content, as well as the I/Cr and I/Rb content ratio increase (Table 4). These characteristics can be used to estimate the "biological age" of the male thyroid gland.

A significant direct correlation between the Ag and Co, Ag and Cr, Co and Cr, Cr and Rb, I and Sb, I and Se, Rb and Sc, Rb and Zn, Sb and Se mass fractions as well as an inverse correlation between I and Fe, Co and Hg, Cr and Hg, Hg and Sc, mass fractions was seen in male thyroid (Table 5).

Since no correlations were found between I and other TE, except for a direct correlation between I and Sb, I and Se an, as well as for inverse correlation between I and Fe, it would appear that the content of Ag, Co, Cr, Hg, Rb, and Zn in the thyroid gland is independent of I content. However, this is not quite so. If we bring the content of the studied TE to the content of I (I/TE ratio), then there are close relationships between I/Ag, I/Co, I/Cr, I/Fe, I/Hg, I/Rb, and I/Zn. (Table 6). From this it follows that, at least, the levels of Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn in the thyroid gland are interconnected and depend on the content of I in it. Because I plays a decisive role in the function of the thyroid gland, the data obtained allow us to conclude that, along with I, such TE as Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn, if not directly, then indirectly, are involved in the process of thyroid hormone synthesis.

Conclusion

The neutron activation analysis is a useful analytical tool for the non-destructive determination of TE contents in the thyroid tissue samples. This method allows at least determine means for Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn (ten TE).

Our data reveal that the I and Se content, as well as the I/Cr and I/Rb content ratio increase in the normal thyroid of male during a lifespan. Therefore, a goitrogenic and tumorogenic effect of excessive I and Se level in the thyroid of old males and of disturbance in intrathyroidal I/Cr and I/Rb relationships with increasing age may be assumed. Furthermore, it was found that the levels of Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn in the thyroid gland are interconnected and depend on the content of I in it. Because I plays a decisive role in the function of the thyroid gland, the data obtained allow us to conclude that, along with I, at least such TEs as Co, Cr, Fe, Rb, Sb, Se, and Zn, if not directly, then indirectly, are involved in the process of thyroid hormone synthesis.

Acknowledgements

We are grateful to Dr. Yu. Choporov, Head of the Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples.

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