ADITUM Journal of Clinical Research and Clinical Case Reports

Open Access Research Article

Comparison between Trace Element Contents in Macro and Micro Follicular Colloid Goiter using Neutron Activation Analysis

Vladimir Zaichick

Radionuclide Diagnostics Department Medical Radiological Research Centre Obninsk, Russia

Article Info

Received: September 02, 2021
Accepted: September 22, 2021
Published: September 24, 2021

***Corresponding author:** Vladimir Zaichick, Medical Radiological Research Centre Korolyev St.4, Obninsk 249036, Kaluga Region, Russia.

Citation: Vladimir Zaichick. "Comparison between Trace Element Contents in Macro and Micro Follicular Colloid Goiter using Neutron Activation Analysis". Clinical Research and Clinical Case Reports, 2(2); DOI: http;//doi.org/04.2021/1.1035.

Abstract

Etiology of colloid nodular goiter (CNG) is unclear. It is known that not only iodine (I) but other trace elements (TE) are involved in goitrogenesis. The present study was performed to clarify the preferential accumulation of some TE either in the colloid or in cells of the thyroid gland. Ten trace elements Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se), and Zn in the thyroid tissues with diagnosed CNG were prospectively evaluated in 16 patients with macro-follicular CNG and 13 patients with micro-follicular CNG. Control group included thyroid tissue samples from 105 healthy individuals. Measurements were performed using neutron activation analysis. It was found that that in macro-follicular CNG the mass fraction of Ag, Hg, and Zn is 12.0, 23.3, and 1.36 times, respectively, higher than in tissues of the normal thyroid. In micro-follicular CNG the mass fraction of Ag, Co, Hg, Rb, Sc, and Zn is 18.2, 1.86, 20.5, 1.33, 1.91, and 1.48 times, respectively, higher than in tissues of the normal thyroid. It was also shown that Co increasingly associated with thyroid cells.

Key words: Macro- and micro follicular colloid nodular goiter of thyroid; Intact thyroid; Trace elements; Instrumental neutron activation analysis.

1. Introduction

Colloid nodular goiter (CNG) is the most common disease of the thyroid, even in non-endemic regions [1]. CNG is clinically detected in about 4% of people older than 30 years [1]. CNG is benign lesion; however, during clinical examination, it can mimic malignant tumors. Furthermore, the origination of CNG can indicate the beginning of malignant transformation of the thyroid gland [2].

Up to now, an etiology of CNG is unclear and probably it is multifactorial [3]. There is opinion that CNG occurs when the thyroid is unable to meet the metabolic demands of the body with sufficient hormone production. The thyroid gland compensates by enlarging, which usually overcomes mild deficiencies of thyroid hormones. For over 20th century, there was the dominant hypothesis that CNG is the simple consequence of iodine (I) deficiency, because I is an essential part of thyroid hormones. However, it was found that CNG is a frequent disease even in those countries and regions where the population is never exposed to I shortage [4]. Moreover, it was shown that I excess has severe consequences on human health and associated with the presence of thyroidal disfunctions and autoimmunity, CNG and diffuse goiter, benign and malignant tumors of gland [5-8]. It was also demonstrated that besides I deficiency and excess many other dietary, environmental, and occupational factors are associated with the CNG incidence [9-11]. Among them a disturbance of evolutionary stable input of many trace elements (TE) in human body after industrial revolution plays a significant role in etiology of thyroidal disorders [12].

Besides I involved in thyroid function, other TE have also essential physiological functions such as maintenance and regulation of cell function, gene regulation, activation or inhibition of enzymatic reactions, and regulation of membrane function [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of TE depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the TE may disturb the cell functions and may result in cellular degeneration, death, benign or malignant transformation [13-15].

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 level in the normal thyroid was investigated in relation to age, gender and some non-thyroidal diseases [23,24]. After that, variations of TE content with age in the thyroid of males and females were studied and age- and gender-dependence of some TE was observed [25-41]. Furthermore, a significant difference between some TE contents in normal and cancerous thyroid was demonstrated [42-47].

Histologically, the CNG is cellular hyperplasia of the thyroid acini. There are two histological types of CNG: macro- and micro-follicular. It is obvious that these two types of CNG have different volume ratios “colloid to cells”.

The present study was performed to clarify the preferential accumulation of some TE either in the colloid or in cells of the thyroid gland. Having this in mind, our aim was to assess the silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), and zinc (Zn) contents in macro- and micro-follicular CNG tissue using instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR). A further aim was to compare the levels of these TE in the macro- and micro-follicular CNG separately with those in intact (normal) gland of apparently healthy persons, as well as to find differences between the levels of these TE in the macro- and micro-follicular CNG.

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

2. Material and Methods

All patients suffered from CNG (n=29, mean age M±SD was 47±14 years, range 30-64) 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. Histological conclusion for all thyroidal lesions was the macro-follicular CNG (n=16) and micro-follicular CNG (n=13).

Normal thyroids for the control group samples were removed at necropsy from 105 deceased (mean age 44±21 years, range 2-87), who had died suddenly. The majority of deaths were due to trauma. A histological examination in the control group was used to control the age norm conformity, as well as to confirm the absence of micro-nodules and latent cancer.

All tissue samples were divided into two portions using a titanium scalpel [48]. 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 [49]. The pounded sample weighing about 5-10 mg (for biopsy) and 50 mg (for resected materials) was used for trace element measurement by INAA-LLR. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

To determine contents of the TE by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [50]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) sub-samples weighing about 50 mg were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results.

A vertical channel of nuclear reactor was applied to determine the content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-LLR. The quartz ampoule with samples of thyroid, standards, and certified reference material was soldered, positioned in a transport aluminum container and exposed to a 24-hour neutron irradiation in a vertical channel of the WWR-c research nuclear reactor (Branch of Karpov Institute, Obninsk) with a neutron flux of $1.3{\cdot}10^{13}$ $n{\cdot}cm^{-2}{\cdot}s^{-1}$. Ten days after irradiation samples were reweighed and repacked.

The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 min to 10 hours subject to pulse counting rate. The gamma spectrometer included the 100 cm^3 Ge(Li) detector and on-line computer-based MCA system. The spectrometer provided a resolution of 1.9 keV on the ^{60}Co 1332 keV line. Details of used nuclear reactions, radionuclides, and gamma-energies were presented in our earlier publications concerning the INAA of TE contents in human prostate and scalp hair [51,52].

A dedicated computer program for INAA mode optimization was used [53]. 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. The difference in the results between normal thyroid and two groups of CNG (separately macro- and micro-follicular), as well as between two groups of CNG was evaluated by the parametric Student’s *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

3. Results

Table 1 depicts our data for Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions in ten sub-samples of IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) certified reference material and the certified values of this material.

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
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 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)

Table 2 presents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Br, Cu, Fe, Rb, Sr, Zn mass fraction in normal thyroid (n=105), macro-follicular CNG (n=16), and micro-follicular CNG (n=13).

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal	Ag	0.0151	0.0140	0.0016	0.0012	0.0800	0.0121	0.0017	0.0454
n=105	Co	0.0399	0.0271	0.0030	0.0046	0.140	0.0327	0.0134	0.124
	Cr	0.539	0.272	0.032	0.130	1.30	0.477	0.158	1.08
	Fe	225	100	11	51.0	512	217	67.4	456
	Hg	0.0421	0.0358	0.0041	0.0065	0.180	0.0304	0.0091	0.150
	Rb	7.37	4.10	0.44	1.11	29.4	6.49	2.60	16.7
	Sb	0.111	0.072	0.008	0.0047	0.308	0.103	0.0117	0.280
	Sc	0.0046	0.0038	0.0008	0.0002	0.0143	0.0042	0.00035	0.0131
	Se	2.32	1.29	0.14	0.439	5.80	2.01	0.775	5.65
	Zn	97.8	42.3	4.5	8.10	221	91.7	34.8	186
Macro-	Ag	0.181	0.242	0.070	0.0020	0.874	0.120	0.0022	0.735
n=16	Co	0.0530	0.0269	0.0072	0.0150	0.101	0.0477	0.0176	0.0974
	Cr	0.612	0.345	0.092	0.135	1.22	0.537	0.174	1.17
	Fe	437	411	103	69.0	1344	211	73.0	1235
	Hg	0.983	0.850	0.227	0.0817	3.01	0.927	0.0876	2.59
	Rb	7.98	3.77	0.94	1.00	15.9	7.40	2.05	14.7
	Sb	0.081	0.072	0.019	0.0102	0.267	0.059	0.0113	0.245
	Sc	0.0068	0.0115	0.0030	0.0002	0.0400	0.0043	0.0002	0.0328
	Se	3.10	2.84	0.73	1.30	12.6	2.05	1.33	10.1
	Zn	133	59	15	87.7	278	109	88.2	275
Micro-	Ag	0.275	0.276	0.087	0.0020	0.842	0.200	0.0140	0.811
n=13	Co	0.0743	0.0201	0.0067	0.0561	0.123	0.0701	0.0568	0.114
	Cr	1.02	1.20	0.40	0.234	3.65	0.617	0.244	3.41
	Fe	218	111	35	98.5	374	161	104	368
	Hg	0.865	0.717	0.227	0.168	2.22	0.565	0.193	2.11
	Rb	9.83	3.14	0.95	6.00	16.6	9.30	6.13	16.0
	Sb	0.143	0.089	0.028	0.050	0.339	0.118	0.050	0.316
	Sc	0.0088	0.0051	0.0020	0.0016	0.0175	0.0071	0.0024	0.0172
	Se	2.59	0.77	0.25	1.50	3.90	2.67	1.52	3.80
	Zn	145	43	12	82.0	235	137	86.0	223

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.

Table 2. Some statistical parameters of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid and colloid nodular goiter of different histology (macro- and micro-follicular)

The comparison of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction in normal thyroid with those in macro- and micro-follicular CNG is shown in Table 3 and 4, respectively.

The ratios of means and the difference between mean values of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions in macro- and micro-follicular CNG are presented in Table 5.

Element	Thyroid tissue				Ratio
	Normal thyroid n=105	Macro-follicular goiter n=16	Student's t-test *p*≤	U-test *p*	Goiter to Norm
Ag	0.0151±0.0016	0.181±0.070	**0.037**	**≤0.01**	12.0
Co	0.0399±0.0030	0.0530±0.0072	0.109	>0.05	1.33
Cr	0.539±0.032	0.612±0.092	0.462	>0.05	1.14
Fe	225±11	437±103	0.057	>0.05	1.94
Hg	0.0421±0.0041	0.983±0.227	**0.001**	**≤0.01**	23.3
Rb	7.37±0.44	7.98±0.94	0.568	>0.05	1.08
Sb	0.111±0.008	0.081±0.019	0.161	>0.05	0.73
Sc	0.0046±0.0008	0.0068±0.0030	0.542	>0.05	1.48
Se	2.32±0.14	3.10±0.73	0.313	>0.05	1.34
Zn	97.8±4.5	133±15	**0.032**	**≤0.01**	1.36

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

Table 3. Differences between mean values (M±SEM) of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid and macro-follicular colloid nodular goiter

Element	Thyroid tissue				Ratio
	Normal thyroid n=105	Micro-follicular goiter n=13	Student's t-test *p*≤	U-test *p*	Goiter to Norm
Ag	0.0151±0.0016	0.275±0.087	**0.015**	**≤0.01**	18.2
Co	0.0399±0.0030	0.0743±0.0067	**0.0006**	**≤0.01**	1.86
Cr	0.539±0.032	1.02±0.40	0.261	>0.05	1.89
Fe	225±11	218±35	0.849	>0.05	0.97
Hg	0.0421±0.0041	0.865±0.227	**0.0055**	**≤0.01**	20.5
Rb	7.37±0.44	9.83±0.95	**0.033**	**≤0.01**	1.33
Sb	0.111±0.008	0.143±0.028	0.298	>0.05	1.29
Sc	0.0046±0.0008	0.0088±0.0020	**0.044**	**≤0.05**	1.91
Se	2.32±0.14	2.59±0.25	0.346	>0.05	1.08
Zn	97.8±4.5	145±12	**0.0042**	**≤0.01**	1.48

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

Table 4. Differences between mean values (M±SEM) of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid and micro-follicular colloid nodular goiter

Element	Thyroid tissue				Ratio
	Macro-follicular goiter n=16	Micro-follicular goiter n=13	Student's t-test *p*≤	U-test *p*	Macro- to Micro
Ag	0.181±0.070	0.275±0.087	0.409	>0.05	0.66
Co	0.0530±0.0072	0.0743±0.0067	**0.042**	**≤0.01**	0.71
Cr	0.612±0.092	1.02±0.40	0.343	>0.05	0.60
Fe	437±103	218±35	0.058	>0.05	2.00
Hg	0.983±0.227	0.865±0.227	0.715	>0.05	1.14

Rb	7.98±0.94	9.83±0.95	0.179	>0.05	0.81
Sb	0.081±0.019	0.143±0.028	0.087	>0.05	0.57
Sc	0.0068±0.0030	0.0088±0.0020	0.614	>0.05	0.77
Se	3.10±0.73	2.59±0.25	0.522	>0.05	1.20
Zn	133±15	145±12	0.548	>0.05	0.92

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

Table 5. Differences between mean values (M±SEM) of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry mass basis) in macro-and micro-follicular colloid nodular goiter

4. Discussion

4.1. Precision and accuracy of results

Good agreement of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents analyzed by INAA-LLR with the certified data of CRM IAEA H-4 and IAEA HH-1 (Table 1) indicates an acceptable accuracy of the results obtained in the study of TE of the thyroid presented in Tables 2–5.

The mean values and all selected statistical parameters were calculated for ten TE (Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn) mass fractions (Table 2). The mass fraction of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn were measured in all, or a major portion of normal thyroid and CNG samples.

4.2. Effect of goitrous transformation on TE contents

From Table 3, it is observed that in macro-follicular CNG the mass fraction of Ag, Hg, and Zn is 12.0, 23.3, and 1.36 times, respectively, higher than in tissues of the normal thyroid. From Table 4, it is observed that in micro-follicular CNG the mass fraction of Ag, Co, Hg, Rb, Sc, and Zn is 18.2, 1.86, 20.5, 1.33, 1.91, and 1.48 times, respectively, higher than in tissues of the normal thyroid. Thus, if we accept the TE contents in thyroid glands in the control group as a norm, we have to conclude that with a goitrous transformation the Ag, Co, Hg, Rb, Sc, and Zn level in thyroid tissue can be significantly changed.

4.3.Association between TE levels and relative volume of colloid and cells

Comparison mass fraction of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn in macro- and micro-follicular CNG shown that level of Co in micro-follicular goiter is 40% higher than in macro-follicular goiter (Table 5). Because the relative volume of cells in the micro-follicular CNG is higher than in the macro-follicular CNG, it is possible to conclude that Co increasingly associated with thyroid cells.

4.4. Comparison with published data

The published data on TE contents in the CNG in comparison with normal levels are very scanty and contradictory. For example, Kovalev [54] found elevated levels of Ag in the CNG, but Gudzhedzhiani [55] did not. A significant decrease of the Zn content during goitrous transformation was shown by Błazewicz et al. [56], but in the recent study this change was not confirmed [9]. Information on the TE contents in macro- or micro-follicular CNG, as well as about the association between TE level and relative volume of colloid and cells in goitrous tissue was not found.

4.5. Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only ten TE (Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of TE investigated in normal and goitrous thyroid. Secondly, the sample size of macro- or micro-follicular CNG groups was relatively small and prevented investigations of TE contents in CNG group using differentials like gender, stage of disease, and dietary habits of healthy persons and patients with CNG. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on goiter-specific tissue of Ag, Co, Hg, Rb, Sc, and Zn level alteration, demonstrates associations between Co content and relative volume of cells in CNG, and shows the necessity to continue TE research of CNG of different histology.

5. Conclusion

In this work, TE analysis was carried out in the tissue samples of normal and goitrous thyroid using INAA-LLR. It was shown that INAA-LLR is an adequate analytical tool for the non-destructive determination of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn content in the tissue samples of human thyroid in norm and pathology, including needle-biopsy cores. It was observed the considerable changes in TE contents in the goitrous transformed tissue of thyroid, which depend on the histology of goiter. It was found that Co predominately accumulates in thyroid cells.

Acknowledgements

The author is extremely grateful to Profs. B.M. Vtyurin and V.S. Medvedev, Medical Radiological Research Center, Obninsk, as well as to Dr. Yu. Choporov, Head of the Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this article.

Funding Sources

None.

References

1. Stuchi LP, Castanhole-Nunes MMU, Maniezzo-Stuchi N,

Biselli-Chicote P, Henrique T, Neto JAP, de-Santi Neto D, Girol AP, Pavarino EC, Goloni-Bertollo EM. EGFA and NFE2L2 gene expression and regulation by microRNAs in thyroid papillary cancer and colloid **g**oiter**.** Genes (Basel) 2020; 11(9): 954.
2. Campbell MJ, Seib CD, Candell L, Gosnell JE, Duh QY, Clark OH, Shen WT. The underestimated risk of cancer in patients with multinodular goiters after a benign fine needle aspiration. World J. Surg 2015; 39: 695–700.
3. Frilling A, Liu C, Weber F. Benign multinodular goiter. Scan J Surg 2004; 93: 278–281.
4. Derwahl M, Studer H. Multinodular goitre: 'much more to it than simply iodine deficiency'. Baillieres Best Pract Res Clin Endocrinol Metab 2000; 14(4): 577-600.
5. Zaichick V. Iodine excess and thyroid cancer. J Trace Elem Exp Med 1998; 11(4): 508-509.
6. Zaichick V., Iljina T. Dietary iodine supplementation effect on the rat thyroid ^{131}I blastomogenic action. In: Die Bedentung der Mengen- und Spurenelemente. 18. Arbeitstangung. Friedrich-Schiller-Universität, Jena, 1998, p. 294-306.
7. Kim S, Kwon YS, Kim JY, Hong KH, Park YK. Association between Iodine Nutrition Status and Thyroid Disease-Related Hormone in Korean Adults: Korean National Health and Nutrition Examination Survey VI (2013-2015). Nutrients 2019; 11(11): 2757.
8. Vargas-Uricoechea P, Pinzón-Fernández MV, Bastidas-Sánchez BE, Jojoa-Tobar E, Ramírez-Bejarano LE, Murillo-Palacios J. Iodine status in the colombian population and the impact of universal salt iodization: a double-edged sword? J Nutr Metab 2019; 2019: 6239243.
9. Stojsavljević A, Rovčanin B, Krstić D, Borković-Mitić S, Paunović I, Diklić A, Gavrović-Jankulović M, Manojlović D. Risk assessment of toxic and essential trace metals on the thyroid health at the tissue level: The significance of lead and selenium for colloid goiter disease. Expo Health 2019.
10. Fahim YA, Sharaf NE, Hasani IW, Ragab EA, Abdelhakim HK. Assessment of thyroid function and oxidative stress state in foundry workers exposed to lead. J Health Pollut 2020; 10(27): 200903.
11. Liu M, Song J, Jiang Y, Lin Y, Peng J, Liang H, Wang C, Jiang J, Liu X, Wei W, Peng J, Liu S, Li Y, Xu N, Zhou D, Zhang Q, Zhang J. A case-control study on the association of mineral elements exposure and thyroid tumor and goiter. Ecotoxicol Environ Saf 2021; 208: 111615.
12. Zaichick V. Medical elementology as a new scientific discipline. J Radioanal Nucl Chem 2006; 269: 303-309.
13. Moncayo R, Moncayo H. A post-publication analysis of the idealized upper reference value of 2.5 mIU/L for TSH: Time to support the thyroid axis with magnesium and iron especially in the setting of reproduction medicine. BBA Clin 2017; 7: 115–119.
14. Beyersmann D, Hartwig A. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 2008; 82(8): 493-512.
15. Martinez-Zamudio R, Ha HC. Environmental epigenetics in metal exposure. Epigenetics 2011; 6(7): 820-827.
16. Zaĭchik VE, Raibukhin YuS, Melnik AD, Cherkashin VI. Neutron-activation analysis in the study of the behavior of iodine in the organism. Med Radiol (Mosk) 1970; 15(1): 33-36.
17. Zaĭchik VE, Matveenko EG, Vtiurin BM, Medvedev VS. Intrathyroid iodine in the diagnosis of thyroid cancer. Vopr Onkol 1982; 28(3): 18-24.
18. Zaichick V, Tsyb AF, Vtyurin BM. Trace elements and thyroid cancer. Analyst 1995; 120(3): 817-821.
19. Zaichick VYe, Choporov YuYa. Determination of the natural level of human intra-thyroid iodine by instrumental neutron activation analysis. J Radioanal Nucl Chem 1996; 207(1): 153-161.
20. Zaichick V. *In vivo* and *in vitro* application of energy-dispersive XRF in clinical investigations: experience and the future. J Trace Elem Exp Med 1998; 11(4): 509-510.
21. Zaichick V, Zaichick S. Energy-dispersive X-ray fluorescence of iodine in thyroid puncture biopsy specimens. J Trace Microprobe Tech 1999; 17(2): 219-232.
22. Zaichick V. Relevance of, and potentiality for in vivo intrathyroidal iodine determination. Ann N Y Acad Sci 2000; 904: 630-632.
23. Zaichick V, Zaichick S. Normal human intrathyroidal iodine. Sci Total Environ 1997; 206(1): 39-56.
24. Zaichick V., Human intrathyroidal iodine in health and non-thyroidal disease. In: New aspects of trace element research (Eds: M.Abdulla, M.Bost, S.Gamon, P.Arnaud, G.Chazot). Smith-Gordon, London, and Nishimura,Tokyo, 1999, p.114-119.
25. Zaichick V, Zaichick S. Age-related changes of some trace element contents in intact thyroid of females investigated by energy dispersive X-ray fluorescent analysis. Trends Geriatr Healthc 2017, 1(1): 31-38.
26. Zaichick V, Zaichick S. Age-related changes of some trace element contents in intact thyroid of males investigated by energy dispersive X-ray fluorescent analysis. MOJ Gerontol Ger 2017; 1(5): 00028.
27. Zaichick V, Zaichick S. Age-related changes of Br, Ca, Cl, I, K, Mg, Mn, and Na contents in intact thyroid of females investigated by neutron activation analysis. Curr Updates Aging 2017; 1: 5.1.
28. Zaichick V, Zaichick S. Age-related changes of Br, Ca, Cl, I, K, Mg, Mn, and Na contents in intact thyroid of males investigated by neutron activation analysis. J Aging Age Relat Dis 2017; 1(1): 1002.
29. Zaichick V, Zaichick S. Age-related changes of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in intact thyroid of females investigated by neutron activation analysis. J Gerontol Geriatr Med 2017; 3: 015.
30. Zaichick V, Zaichick S. Age-related changes of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in intact thyroid of males investigated by neutron activation analysis. Curr Trends Biomedical Eng Biosci 2017; 4(4): 555644.
31. Zaichick V, Zaichick S. Effect of age on chemical element contents in female thyroid investigated by some nuclear analytical methods. MicroMedicine 2018; *6*(1): 47-61.
32. Zaichick V, Zaichick S. Neutron activation and X-ray fluorescent analysis in study of association between age and chemical element contents in thyroid of males. Op Acc J Bio Eng Bio Sci 2018; 2(4): 202-212.
33. Zaichick V, Zaichick S. Variation with age of chemical element contents in females' thyroids investigated by neutron activation analysis and inductively coupled plasma atomic emission spectrometry. J Biochem Analyt Stud 2018; 3(1): 1-

10.
34. Zaichick V, Zaichick S. Association between age and twenty chemical element contents in intact thyroid of males. SM Gerontol Geriatr Res 2018; 2(1): 1014.
35. Zaichick V, Zaichick S. Associations between age and 50 trace element contents and relationships in intact thyroid of males. Aging Clin Exp Res 2018; 30(9): 1059–1070.
36. Zaichick V, Zaichick S. Possible role of inadequate quantities of intra-thyroidal bromine, rubidium and zinc in the etiology of female subclinical hypothyroidism. EC Gynaecology 2018; 7(3): 107-115.
37. Zaichick V, Zaichick S. Possible role of inadequate quantities of intra-thyroidal bromine, calcium and magnesium in the etiology of female subclinical hypothyroidism. Int Gyn and Women's Health 2018; 1(3): IGWHC.MS.ID.000113.
38. Zaichick V, Zaichick S. Possible role of inadequate quantities of intra-thyroidal cobalt, rubidium and zinc in the etiology of female subclinical hypothyroidism. Womens Health Sci J 2018; 2(1): 000108.
39. Zaichick V, Zaichick S. Association between female subclinical hypothyroidism and inadequate quantities of some intra-thyroidal chemical elements investigated by X-ray fluorescence and neutron activation analysis. Gynaecology and Perinatology 2018; 2(4): 340-355.
40. Zaichick V, Zaichick S. Investigation of association between the high risk of female subclinical hypothyroidism and inadequate quantities of twenty intra-thyroidal chemical elements. Clin Res: Gynecol Obstet 2018; 1(1): 1-18.
41. Zaichick V, Zaichick S. Investigation of association between the high risk of female subclinical hypothyroidism and inadequate quantities of intra-thyroidal trace elements using neutron activation and inductively coupled plasma mass spectrometry. Acta Scientific Medical Sciences 2018; 2(9): 23-37.
42. Zaichick V. Zaichick S. Trace element contents in thyroid cancer investigated by energy dispersive X-ray fluorescent analysis. American Journal of Cancer Research and Reviews 2018; 2: 5.
43. Zaichick V., Zaichick S. Trace element contents in thyroid cancer investigated by instrumental neutron activation analysis. J Oncol Res 2018; 2(1): 1-13.
44. Zaichick V, Zaichick S. Variation in selected chemical element contents associated with malignant tumors of human thyroid gland. Cancer Studies 2018; 2(1): 2
45. Zaichick V, Zaichick S. Twenty chemical element contents in normal and cancerous thyroid. Int J Hematol Blo Dis 2018; 3(2): 1-13.
46. Zaichick V, Zaichick S. Levels of chemical element contents in thyroid as potential biomarkers for cancer diagnosis (a preliminary study). J Cancer Metastasis Treat 2018; 4: 60.
47. Zaichick V, Zaichick S. Fifty trace element contents in normal and cancerous thyroid. Acta Scientific Cancer Biology 2018; 2(8): 21-38.
48. Zaichick V, Zaichick S. Instrumental effect on the contamination of biomedical samples in the course of sampling. The Journal of Analytical Chemistry 1996; 51(12): 1200-1205.
49. Zaichick V, Zaichick S. A search for losses of chemical elements during freeze-drying of biological materials. J Radioanal Nucl Chem 1997; 218(2): 249-253.
50. Zaichick V. Applications of synthetic reference materials in the medical Radiological Research Centre. Fresenius J Anal Chem 1995; 352: 219-223.
51. Zaichick S, Zaichick V. The effect of age on Ag, Co, Cr, Fe, Hg, Sb, Sc, Se, and Zn contents in intact human prostate investigated by neutron activation analysis. J Appl Radiat Isot 2011; 69: 827-833.
52. Zaichick S, Zaichick V. The effect of age and gender on 37 chemical element contents in scalp hair of healthy humans. Biol Trace Elem Res 2010; 134(1): 41-54.
53. Korelo AM, Zaichick V. Software to optimize the multielement INAA of medical and environmental samples. In: Activation Analysis in Environment Protection. Joint Institute for Nuclear Research, Dubna, Russia, 1993, p. 326-332.
54. Kovalev MM. Trace element contents in normal and goitrous glands. Vrach Delo 1960; 12: 107-111.
55. Gudzhedzhiani RB. About copper, zinc, lead, and silver in blood and thyroid tissue of patients with thyrotoxicosis. PhD thesis, Tbilisi medical institute, Tbilisi 1973.
56. Błazewicz A, Dolliver W, Sivsammye S, Deol A, Randhawa R, Orlicz-Szczesna G, Błazewicz R. Determination of cadmium, cobalt, copper, iron, manganese, and zinc in thyroid glands of patients with diagnosed nodular goitre using ion chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2010; 878(1): 34-38.