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Evaluation of Trace Elements in Thyroid Adenomas Using Energy Dispersive X-Ray Fluorescent Analysis

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ABSTRACT

Thyroid adenomas (TA) are benign tumors, but there is a 20% possibility of malignant transformation. The distinguishing between the TA and thyroid cancer (TC) is tricky, therefore new TA biomarkers are needed. Furthermore, the role of trace elements (TE) in etiology and pathogenesis of TA is unclear. The aim of this exploratory study was to examine the content of bromine (Br), cooper (Cu), iron (Fe), rubidium (Rb), strontium (Sr), and zinc (Zn) in the normal and in adenomatous thyroid. Thyroid tissue levels of six TE were prospectively evaluated in 19 patients with TA and 105 healthy inhabitants. Measurements were performed using ^{109}Cd radionuclide-induced energy-dispersive X-ray fluorescent analysis Tissue samples were divided into two portions. One was used for morphological study while the other was intended for TE analysis. It was found that contents of Br and Cu were significantly higher (25.8 and 4.16 times, respectively) and content of Sr were significantly lower (39%) in adenomatous thyroid in comparison with normal level. There are considerable changes in TE contents in the adenomatous thyroid.

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Introduction

Thyroid adenomas (TA) are homogenous, solitary, encapsulated benign tumors, more common in females, and have a good prognosis [1]. However, because there is a 20% possibility of malignant transformation, TA should be differentiated from other thyroid nodular diseases such as nodular goiter (NG) and thyroid cancer (TC). The distinguishing between the TA and TC is tricky, therefore new differential diagnostics and TA biomarkers are needed [2,3].

For over 20th century, there was the dominant opinion that NG, including TA, is the simple consequence of iodine deficiency. However, it was found that NG is a frequent disease even in those countries and regions where the population is never exposed to iodine shortage [4]. Moreover, it was shown that iodine excess has severe consequences on human health and associated with the presence of thyroidal disfunctions and autoimmunity, NG and diffuse goiter, benign and malignant tumors of gland [5-8]. It was also demonstrated that besides the iodine deficiency and excess many other dietary, environmental, and occupational factors are associated with the NG incidence [9-11]. Among them a disturbance of evolutionary stable input of many chemical elements in human body after industrial revolution plays a significant role in etiology of thyroidal disorders [12].

Besides iodine involved in thyroid function, other trace elements (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. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of TE depend on tissue-specific need or tolerance, respectively. 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]. In our previous studies the complex of in vivo and in vitro nuclear analytical and related methods was developed and used for the investigation of iodine and other TE contents in the normal and pathological thyroid [16-22]. Iodine level in the normal thyroid was investigated in relation to age, gender and some non-thyroidal diseases [23,24]. After that, variations of many 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].

To date, the etiology and pathogenesis of TA has to be considered as multifactorial. The present study was performed to clarify the role of some TE in the TA etiology. Having this in mind, our aim was to assess the bromine (Br), copper (Cu), iron (Fe), rubidium (Rb), strontium (Sr), and zinc (Zn) contents in TA tissue using non-destructive energy dispersive X-ray fluorescent analysis (EDXRF). A further aim was to compare the levels of these TE in the adenomatous thyroid with those in intact (normal) gland of apparently healthy persons.

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.

Material and Methods

All patients suffered from TA (n=19, 16 females and 3 males, mean age M±SD was 41±11 years, range 22-55) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre. Thick-needle puncture biopsy of suspicious nodules of the thyroid was performed for every patient, to permit morphological study of thyroid tissue at these sites and to estimate their TE contents. For all patients the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. Histological conclusion for all thyroidal lesions was the TA.

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 8 mg was applied to the piece of Scotch tape serving as an adhesive fixing backing.

To determine the contents of the TE by comparison with known data for standard, aliquots of commercial, chemically pure compounds and synthetic reference materials were used [50]. The microliter standards were placed on disks made of thin, ash-free filter papers fixed on the Scotch tape pieces and dried in a vacuum. Ten subsamples of the Certified Reference Material (CRM) IAEA H-4 (animal muscle) weighing about 8 mg were analyzed to estimate the precision and accuracy of results. The CRM IAEA H-4 subsamples were prepared in the same way as the samples of dry homogenized thyroid tissue.

Details of the relevant facility for EDXRF, source with ^{109}Cd radionuclide, methods of analysis and the quality control of results were presented in our earlier publications concerning the EDXRF analysis of human thyroid and prostate tissue [25,26,51].

All thyroid samples were prepared in duplicate, and mean values of TE contents were used in final calculation. Using Microsoft Office Excel software, 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 in normal and TA tissue. The difference in the results between two groups (normal thyroid and TA) was evaluated by the parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

Results

Table 1 depicts our data for six TE in ten sub-samples of CRM IAEA H-4 (animal muscle) and the certified values of this material.

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 and adenomatous thyroid.

The comparison of our results with published data for Br, Cu, Fe, Rb, Sr, and Zn mass fraction in normal and adenomatous thyroid [9,52-62] is shown in Table 3.

The ratios of means and the difference between mean values of Br, Cu, Fe, Rb, Sr, Zn mass fractions in normal and adenomatous thyroid are presented in Table 4.

Discussion

Precision and Accuracy of Results

Good agreement of the Br, Cu, Fe, Rb, Sr, and Zn contents analyzed by EDXRF with the certified data of CRM IAEA H-4 (Table 1) indicates an acceptable accuracy of the results obtained in the study of TE of the thyroid samples presented in Tables 2-4.

Table 1: EDXRF data Br, Cu, Fe, Rb, Sr, and Zn contents in the IAEA H-4 (animal muscle) reference material compared to certified values (mg/kg, dry mass basis)

Element	Certified values			This work results
	Mean	95% confidence interval	Type	Mean±SD
Br	4.1	3.5 - 4.7	C	5.0±1.2
Cu	4.0	3.6 - 4.3	C	3.9±1.1
Fe	49	47 - 51	C	48±9
Rb	18	17 - 20	C	22±4
Sr	0.1	-	N	<1
Zn	86	83 - 90	C	90±5

Mean – arithmetical mean, SD – standard deviation, C- certified values, N – non-certified values.

The mean values and all selected statistical parameters were calculated for six trace elements (Br, Cu, Fe, Rb, Sr, and Zn) mass fractions (Table 2). The mass fraction of Br, Cu, Fe, Rb, Sr, and Zn were measured in all, or a major portion of normal and adenomatous tissue samples.

Table 2: Some statistical parameters of Br, Cu, Fe, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal and adenomatous thyroid

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal n=105	Br	13.9	12.0	1.3	1.4	54.4	10.0	2.23	50.8
	Cu	4.23	1.52	0.18	0.50	7.50	4.15	1.57	7.27
	Fe	222	102	11	47.1	512	204	65.7	458
	Rb	9.03	6.17	0.66	1.80	42.9	7.81	2.48	25.5
	Sr	4.55	3.22	0.37	0.10	13.7	3.70	0.48	12.3
	Zn	112	44.0	4.7	6.10	221	106	35.5	188
Adenoma n=19	Br	358	394	118	3.2	1080	189	5.3	1028
	Cu	17.6	14.0	5.7	4.1	35.2	13.8	4.28	35.0
	Fe	262	224	62	52	815	203	52.1	735
	Rb	8.02	3.53	0.91	1.10	14.3	8.30	1.56	13.7
	Sr	2.78	2.04	0.55	0.42	6.66	2.26	0.54	6.47
	Zn	120	55	14	48.0	251	105	52.9	226

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.

Comparison with Published Data

In a general sense values obtained for Br, Cu, Fe, Rb, Sr, and Zn contents in the normal thyroid (Table 3) agree well with median of mean values reported by other researches [52-58]. 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%) [63] and ash (4.16% on dry mass basis) [64] contents in thyroid of adults.

Table 3: Median, minimum and maximum value of means Br, Cu, Fe, Rb, Sr, and Zn contents in normal and adenomatous thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis)

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)**	M±SD
Normal	Br	18.1 (11)	5.12 (44) [52]	284±44 (14) [53]	13.9±12.0
	Cu	5.94 (61)	0.16 (83) [54]	220±22 (10) [55]	4.23±1.52
	Fe	252 (21)	56 (120) [56]	2444±700 (14) [53]	222±102
	Rb	12.3 (9)	≤0.85 (29) [57]	294±191 (14) [53]	9.03±6.17
	Sr	0.61 (9)	0.055 (83) [54]	46.8±4.8 (4) [55]	4.55±3.22
	Zn	118 (55)	1.08 (120) [58]	820±204 (14) [53]	112±44
Adenoma	Br	-	-	-	358±394
	Cu	11.0 (7)	1.24 (46) [9]	29 (5) [59]	17.6±14.0
	Fe	92.5 (4)	15 (5) [59]	2100±208 (4) [55]	262±224
	Rb	7.0 (1)	7,0 (10) [60]	7,0 (10) [60]	8.02±3.53
	Sr	27.2 (1)	27.2±2.4 (4)[55]	27.2±2.4 (4)[55]	2.78±2.04
	Zn	68.5 (8)	21.0 (2) [61]	330±282 (9) [62]	120±55

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

Data cited in Table 3 for normal thyroid also includes samples obtained from patients who died from different non-endocrine diseases. In our previous study it was shown that some non-endocrine diseases can effect on TE contents in thyroid [24]. Moreover, in many studies the "normal" thyroid means a visually non-affected tissue adjacent to benign or malignant thyroidal nodules. However, there are no data on a comparison between the TE contents in such kind of samples and those in thyroid of healthy persons, which permits to confirm their identity.

In adenomatous tissues (Table 3) our results were comparable with published data for Cu, Fe, Rb, and Zn contents. The obtained in present work mean for Sr was one order of magnitude lower the only previously reported result (Table 3). The data on Br level in adenomatous thyroid tissue were not found in the literature.

The range of means of Br, Cu, Fe, Rb, Sr, and Zn level reported in the literature for normal and adenomatous thyroid vary widely (Table 3). This can be explained by a dependence of TE content on many factors, including "normality" of thyroid samples (see above), the region of the thyroid, from which the sample was taken, age, gender, ethnicity, and mass of the gland, as well as the TA stage, histology, and functional activity. Not all these factors were strictly controlled in cited studies. However, in our opinion, the leading causes of inter-observer variability can be attributed to the accuracy of the analytical techniques, sample preparation methods, and inability of taking uniform samples from the affected tissues. It was insufficient quality control of results in these studies. In many scientific reports, tissue samples were ashed or dried at high temperature for many hours. In other cases, thyroid samples were treated with solvents (distilled water, ethanol, formalin etc). There is evidence that during ashing, drying and digestion at high temperature some quantities of certain TE are lost as a result of this treatment. That concerns not only such volatile halogen as Br, but also other TE investigated in the study [65,66].

Effect of Adenomatous Transformation on TE Contents

From Table 4, it is observed that in adenomatous tissue the mass fraction of Sr is 39% lower whereas mass fractions of Br and Cu are 25.8 and 4.16 times, respectively, higher than in normal tissues of the thyroid. Thus, if we accept the TE contents in thyroid glands in the control group as a norm, we have to conclude that with an adenomatous transformation the Br, Cu, and Sr level in thyroid tissue significantly changed.

Table 4: Differences between mean values (M±SEM) of Br, Cu, Fe, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal and adenomatous thyroid

Element	Thyroid tissue				Ratio
	Norm n=105	Adenoma n=19	Student's t-test $p\leq$	U-test p	
Br	13.9±1.3	358±118	**0.016**	**≤0.01**	25.8
Cu	4.23±0.18	17.6±5.7	**0.066**	**≤0.05**	4.16
Fe	222±11	262±62	0.533	>0.05	1.18
Rb	9.03±0.66	8.02±0.91	0.375	>0.05	0.89
Sr	4.55±0.37	2.78±0.55	**0.013**	**≤0.01**	0.61
Zn	112±5	120±14	0.621	>0.05	1.07

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

Role of Trace Elements in Adenomatous Transformation of the Thyroid

Characteristically, elevated or reduced levels of TE observed in adenomatous tissues are discussed in terms of their potential role in the initiation and promotion of TA. In other words, using the low or high levels of the TE in adenomatous tissues researchers try to determine the role of the deficiency or excess of each TE in the TA etiology. In our opinion, abnormal levels of many TE in TA could be and cause, and also effect of adenomatous transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in TE level in pathologically altered tissue is the reason for alterations or vice versa.

Bromine

This is one of the most abundant and ubiquitous of the recognized ChE in the biosphere. Inorganic bromide is the ionic form of bromine which exerts therapeutic as well as toxic effects. An enhanced intake of bromide could interfere with the metabolism of iodine at the whole-body level. In the thyroid gland the biological behavior of bromide is more similar to the biological behavior of iodide [67]. Moreover, many studies indicate that bromate (BrO^{3-}) and potassium bromate (KB_rO_3) are carcinogens. Bromate is formed as a drinking water ozone disinfection by-product and also used in some food and consumer product. Potassium bromate is a chemical oxidizing agent that used extensively in food and cosmetic industries [68-70]. Potassium bromate is also found in drinking water as a disinfection by-product of surface water ozonation [68].

In our previous studies it was found a significant age-related increase of Br content in human thyroid [25-28]. This finding correlated with a significant age-related increase of thyroid cancer incidents. Furthermore, elevated levels of Br in cancerous thyroid and malignant tumor of prostate were indicated [42-47,71-74].

Thus, on the one hand, the accumulated data suggest that Br might be responsible for TA development. But, on the other hand, Br compounds, especially potassium bromide (KBr), sodium bromide (NaBr), and ammonium bromide (NH_4Br), are frequently used as sedatives in Russia [75]. It may be the reason for elevated levels of Br in specimens of patients with TA. Anyway, the accumulation of Br in adenomatous thyroids could possibly be explored for diagnosis of TA.

Copper

This is a ubiquitous element in the human body which plays many roles at different levels. Various Cu-enzymes (such as amine oxidase, ceruloplasmin, cytochrome-c oxidase, dopamine-monooxygenase, extracellular superoxide dismutase, lysyl oxidase, peptidylglycineamidating monoxygenase, Cu/Zn superoxide dismutase, and tyrosinase) mediate the effects of Cu deficiency or excess. Cu excess can have severe negative impacts. Cu generates oxygen radicals and many investigators have hypothesized that excess copper might cause cellular injury via an oxidative pathway, giving rise to enhanced lipid peroxidation, thiol oxidation, and, ultimately, DNA damage [76-78]. Thus, Cu accumulation in thyroid parenchyma with age may be involved in oxidative stress, dwindling gland function, and increasing risk of thyroid nodules, including TA and TC [25,26]. The significantly elevated level of Cu in thyroid adenomatous tissue, observed in the present study, supports this speculation. However, an overall comprehension of Cu homeostasis and physiology, which is not yet acquired,

is mandatory to establish Cu exact role in the TA etiology and metabolism. Anyway, the accumulation of Cu in adenomatous thyroids could possibly be explored for diagnosis of TA.

Representative literature data on the Cu content in TA are limited. Moreover, there are great contradictions in the results between the reported studies. For example, Koch et al. [61] reported that the content of Cu was higher in adenomatous tissues compared with that in normal thyroid. These data are in good agreement with our results. The completely opposite result was demonstrated by Reddy et al. [55]. In recent study [9] no difference between Cu level in normal and adenomatous thyroid was found.

Strontium

The role of Sr in the thyroid function and TA etiology is unknown. We can't explain why the Sr level in adenomatous tissues is almost twice lower than in normal thyroid. Interestingly remark, however, that very similar result of reduced Sr content in TA was indicated in the only published article on the subject [55]. Anyway, the significantly reduced level of Sr in adenomatous thyroids could possibly be explored for diagnosis of TA.

Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only six TE (Br, Cu, Fe, Rb, Sr, 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 adenomatous thyroid. Secondly, the sample size of TA group was relatively small and prevented investigations of TE contents in TA group using differentials like gender, histological types of adenoma and its functional activity, stage of disease, dietary habits of healthy persons and patients with TA. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on adenoma-specific tissue Br, Cu, and Sr level alteration and shows the necessity to continue TE research of TA.

Conclusion

In this work, TE analysis was carried out in the tissue samples of normal and adenomatous thyroid using EDXRF. It was shown that EDXRF is an adequate analytical tool for the non-destructive determination of Br, Cu, Fe, Rb, Sr, and Zn content in the tissue samples of human thyroid in norm and pathology, including needle-biopsy cores. It was observed that in adenomatous tissues content of Sr was significantly lower, while contents of Br and Cu were significantly higher than in normal tissues. In our opinion, the abnormal decrease in level of Sr, as well as the increase in levels of Br, and Cu in adenomatous tissue might demonstrate an involvement of these TE in etiology and pathogenesis of TA. It was supposed that elevated levels of Br and Cu, as well as reduced level of Sr in thyroid affected tissue can be used as TA markers.

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References

1. Welker MJ, Orlov D (2003) Thyroid Nodules. Am Fam Physician 67: 559-567.
2. Kant R, Davis A, Verma V (2020) Thyroid nodules: Advances in evaluation and management. Am Fam Physician 102: 298-304.
3. Sato A, Matsuda K, Motoyama T, Mussazhanova Z, Otsubo R et al. (2021) 53BP1 expression as a biomarker to differentiate thyroid follicular tumors 10: 309-315.
4. Derwahl M, Studer H. Multinodular goiter (200) 'much more to it than simply iodine deficiency'. Baillieres Best Pract Res Clin Endocrinol Metab 14: 577-600.
5. Zaichick V (1998) Iodine excess and thyroid cancer. J Trace Elem Exp Med 11: 508-509.
6. Zaichick V, Iljina T (1998) Dietary iodine supplementation effect on the rat thyroid 131I blastomogenic action. In: Die Bedentung der Mengen- und Spurenelemente. 18. Arbeitstangung. Friedrich-Schiller-Universität, Jena 294-306.
7. Kim S, Kwon YS, Kim JY, Hong KH, Park YK (2019) Association between iodine nutrition status and thyroid disease-related hormone in Korean adults: Korean National Health and Nutrition Examination Survey VI (2013-2015). Nutrients 11: 2757.
8. Vargas-Uricoechea P, Pinzón-Fernández MV, Bastidas-Sánchez BE, Jojoa-Tobar E, Ramírez-Bejarano LE et al. (2019) Iodine status in the colombian population and the impact of universal salt iodization: a double-edged sword? J Nutr Metab 2019: 6239243.
9. Stojsavljević A, Rovčanin B, Krstić D, Borković-Mitić S, Paunović I, et al. (2019) 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.
10. Fahim YA, Sharaf NE, Hasani IW, Ragab EA, Abdelhakim HK et al. (2020) Assessment of thyroid function and oxidative stress state in foundry workers exposed to lead. J Health Pollut 10: 200903.
11. Liu M, Song J, Jiang Y, Lin Y, Peng J et al. (2021) case-control study on the association of mineral elements exposure and thyroid tumor and goiter. Ecotoxicol Environ Saf 208: 111615.
12. Zaichick V (2006) Medical elementology as a new scientific discipline. J Radioanal Nucl Chem 269: 303-309.
13. Moncayo R, Moncayo H (2017) 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 7: 115-119.
14. Beyersmann D, Hartwig A (2008) Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms 82: 493-512.
15. Martinez-Zamudio R, Ha HC (2011) Environmental epigenetics in metal exposure. 6: 820-827.
16. Zaĭchik VE, Raibukhin YuS, Melnik AD, Cherkashin VI (1970) Neutron-activation analysis in the study of the behavior of iodine in the organism. Med Radiol (Mosk) 15: 33-36.
17. Zaĭchik VE, Matveenko EG, Vtiurin BM, Medvedev VS (1982) Intrathyroid iodine in the diagnosis of thyroid cancer. Vopr Onkol 28: 18-24.
18. Zaichick V, Tsyb AF, Vtyurin BM (1995) Trace elements and thyroid cancer. Analyst 120: 817-821.
19. Zaichick VYe, Choporov YuYa (1996) Determination of the natural level of human intra-thyroid iodine by instrumental neutron activation analysis. J Radioanal Nucl Chem 207: 153-161.
20. Zaichick V (1998) In vivo and in vitro application of energy-dispersive XRF in clinical investigations: experience and the future. J Trace Elem Exp Med 11: 509-510.
21. Zaichick V, Zaichick S (1999) Energy-dispersive X-ray fluorescence of iodine in thyroid puncture biopsy specimens. J Trace Microprobe Tech 17: 219-232.
22. Zaichick V (2000) Relevance of, and potentiality for in vivo intrathyroidal iodine determination. Ann N Y Acad Sci 904:

630-632.
23. Zaichick V, Zaichick S (1997) Normal human intrathyroidal iodine. Sci Total Environ 206: 39-56.
24. Zaichick V (1999) 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 114-119.
25. Zaichick V, Zaichick S (2017) Age-related changes of some trace element contents in intact thyroid of females investigated by energy dispersive X-ray fluorescent analysis. Trends Geriatr Healthc 1: 31-38.
26. Zaichick V, Zaichick S(2017) Age-related changes of some trace element contents in intact thyroid of males investigated by energy dispersive X-ray fluorescent analysis. MOJ Gerontol Ger 1: 00028.
27. Zaichick V, Zaichick S (2017) 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 1: 5.1.
28. Zaichick V, Zaichick S (2017) 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 1: 1002.
29. Zaichick V, Zaichick S (2017) 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 3: 015.
30. Zaichick V, Zaichick S (2017) 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 4: 555644.
31. Zaichick V, Zaichick S (2018) Effect of age on chemical element contents in female thyroid investigated by some nuclear analytical methods. MicroMedicine 6: 47-61.
32. Zaichick V, Zaichick S (2018) 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 2: 202-212.
33. Zaichick V, Zaichick S (2018) 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 3: 1-10.
34. Zaichick V, Zaichick S (2018) Association between age and twenty chemical element contents in intact thyroid of males. SM Gerontol Geriatr Res 2: 1014.
35. Zaichick V, Zaichick S (2018) Associations between age and 50 trace element contents and relationships in intact thyroid of males. Aging Clin Exp Res 30: 1059-1070.
36. Zaichick V, Zaichick S (2018) Possible role of inadequate quantities of intra-thyroidal bromine, rubidium and zinc in the etiology of female subclinical hypothyroidism. EC Gynaecology 7: 107-115.
37. Zaichick V, Zaichick S (2018) 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 1(3): IGWHC.MS.ID.000113.
38. Zaichick V, Zaichick S (2018) Possible role of inadequate quantities of intra-thyroidal cobalt, rubidium and zinc in the etiology of female subclinical hypothyroidism. Womens Health Sci J 2: 000108.
39. Zaichick V, Zaichick S (2018) 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 2: 340-355.
40. Zaichick V, Zaichick S (2018) Investigation of association between the high risk of female subclinical hypothyroidism and inadequate quantities of twenty intra-thyroidal chemical elements. Clin Res: Gynecol Obstet 1: 1-18.
41. Zaichick V, Zaichick S (2018) 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 2: 23-37.
42. Zaichick V. Zaichick S (2018) Trace element contents in thyroid cancer investigated by energy dispersive X-ray fluorescent analysis 2: 5.
43. Zaichick V, Zaichick S (2018) Trace element contents in thyroid cancer investigated by instrumental neutron activation analysis. J Oncol Res 2: 1-13.
44. Zaichick V, Zaichick S (2018) Variation in selected chemical element contents associated with malignant tumors of human thyroid gland. Cancer Studies 2: 2
45. Zaichick V, Zaichick S (2018) Twenty chemical element contents in normal and cancerous thyroid. Int J Hematol Blo Dis 3: 1-13.
46. Zaichick V, Zaichick S (2018) Levels of chemical element contents in thyroid as potential biomarkers for cancer diagnosis (a preliminary study). J Cancer Metastasis Treat 4: 60.
47. Zaichick V, Zaichick S (2018) Fifty trace element contents in normal and cancerous thyroid. Acta Scientific Cancer Biology 2: 21-38.
48. Zaichick V, Zaichick S (1996) Instrumental effect on the contamination of biomedical samples in the course of sampling. The Journal of Analytical Chemistry 51: 1200-1205.
49. Zaichick V, Zaichick S (1997) A search for losses of chemical elements during freeze-drying of biological materials. J Radioanal Nucl Chem 218: 249-253.
50. Zaichick V (1995) Applications of synthetic reference materials in the medical Radiological Research Centre. Fresenius J Anal Chem 352: 219-223.
51. Zaichick S, Zaichick V (2011) The Br, Fe, Rb, Sr, and Zn contents and interrelation in intact and morphologic normal prostate tissue of adult men investigated by energy-dispersive X-ray fluorescent analysis. X-Ray Spectrom 40: 464-469.
52. Zhu H, Wang N, Zhang Y, Wu Q, Chen R, et al. (2010) Element contents in organs and tissues of Chinese adult men. Health Phys 98: 61-73.
53. Salimi J, Moosavi K, Vatankhah S, Yaghoobi A (2004) Investigation of heavy trace elements in neoplastic and non-neoplastic human thyroid tissue: A study by proton – induced X-ray emissions. Iran J Radiat Res 1: 211-216.
54. Reitblat MA, Kropachyev AM (1967) Some trace elements in thyroid of the Perm Pricam'ya residents. Proceedings of Perm Medical Institute 78: 157-164.
55. Reddy SB, Charles MJ, Kumar MR, Reddy BS, Anjaneyulu Ch, et al. (2002) Trace elemental analysis of adenoma and carcinoma thyroid by PIXE method. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 196: 333-339.
56. Ataullachanov IA (1969) Age changes in the content of manganese, cobalt, copper, zinc and iron in the endocrine glands of women. Probl Endocrinol (Mosk) 15: 98-102.
57. Boulyga SF, Zhuk IV, Lomonosova EM, Kanash NV, Bazhanova NN (1997) Determination of microelements in

thyroids of the inhabitants of Belarus by neutron activation analysis using the k0-method. J Radioanal Nucl Chem 222: 11-14.

58. Vlasova ZA (1969) Trace element dynamics in thyroid in connection with age and atherosclerosis. Proceedings of Leningradskii Institute of Medical Doctor Postgraduate Education 80: 135-144.
59. Maeda K, Yokode Y, Sasa Y, Kusuyama H, Uda M (1987) Multielemental analysis of human thyroid glands using particle induced X-ray emission (PIXE). Nucl Instrum Methods Phys Res B 22: 188-190.
60. Kvicala J, Havelka J, Nemec J, Zeman V (1992) Selenium and rubidium changes in subjects with pathologically altered thyroid. Biol Trace Elem Res 32: 253-258.
61. Koch HJ, Smith ER (1956) The determination of copper and zinc in normal and pathologic thyroid tissue. J Clin Endocrinol 16: 123-129.
62. Zagrodzki P, Nicol F, Arthur JR, Słowiaczek M, Walas S, et al. (2010) Biol Trace Elem Res 134: 25-40.
63. Katoh Y, Sato T, Yamamoto Y (2002) Determination of multielement concentrations in normal human organs from the Japanese. Biol Trace Elem Res 90: 57-70.
64. Schroeder HA, Tipton IH, Nason AP (1972) Trace metals in man: strontium and barium. J Chron Dis 25: 491-517.
65. Zaichick V (1997) Sampling, sample storage and preparation of biomaterials for INAA in clinical medicine, occupational and environmental health. In: Harmonization of Health-Related Environmental Measurements Using Nuclear and Isotopic Techniques. IAEA, Vienna 123-133.
66. Zaichick V (2004) Losses of chemical elements in biological samples under the dry aching process. Trace Elements in Medicine 5: 17-22.
67. Pavelka S (2016) Radiometric determination of thyrotoxic effects of some xenobiotics. Rad Applic 1: 155-158.
68. Jahan BN, Li L, Pagilla KR (2021) Fate and reduction of bromate formed in advanced water treatment ozonation systems: A critical review. Chemosphere 266: 128964.
69. Kurokawa Y, Maekawa A, Takahashi M, Hayashi Y (1990) Toxicity and carcinogenicity of potassium bromate—a new renal carcinogen. Environ Health Perspect 87: 309-335.
70. Chipman J, Davies J, Parsons J, Nair J, O'Neill G et al. (1998) DNA oxidation by potassium bromate; a direct mechanism or linked to lipid peroxidation? Toxicology 126: 93–102.
71. Zaichick V, Zaichick S (2016) Trace element contents in adenocarcinoma of human prostate investigated by energy dispersive X-ray fluorescent analysis. Journal of Adenocarcinoma 1: 1-7.
72. Zaichick V, Zaichick S (2016) Trace element contents in adenocarcinoma of the human prostate gland investigated by neutron activation analysis. Cancer Research & Oncology 1: 1-10.
73. Zaichick V, Zaichick S (2016) The Comparison between the contents and interrelationships of 17 chemical elements in normal and cancerous prostate gland. Journal of Prostate Cancer 1: 105.
74. Zaichick V (2017) Differences between 66 chemical element contents in normal and cancerous prostate 6: 37-56.
75. Maschkovsky MD (2005) The sedatives. In: The Medicaments, 15th Ed., Novaya Volna, Moscow 72-86.
76. Li Y, Trush MA (1993) DNA damage resulting from the oxidation of hydroquinone by copper: role for a Cu(II)/Cu(I) redox cycle and reactive oxygen generation. Carcinogenesis 14: 1303-1311.
77. Becker TW, Krieger G, Witte I (1996) DNA single and double strand breaks induced by aliphatic and aromatic aldehydes in combination with copper (II). Free Radic Res 24: 325-332.
78. Glass GA, Stark AA (1997) Promotion of glutathione-gamma-glutamyl transpeptidase-dependent lipid peroxidation by copper and ceruloplasmin: the requirement for iron and the effects of antioxidants and antioxidant enzymes. Environ Mol Mutagen 29: 73-80.