International Journal of Integrated Medical Research
Volume 8 Issue 04 October-November-December 2021
https://doi.org/10.57181/ijoimr/vol8i04/33
ISSN (Online): 2393-9869, ISSN (Print): 2350-0360

RESEARCH ARTICLE

EVALUATION OF THYROID TRACE ELEMENTS IN HASHIMOTO'S THYROIDITIS USING METHOD OF X-RAY FLUORESCENCE

Prof. Dr. Vladimir Zaichick
Radionuclide Diagnostics Department Medical Radiological Research Centre Obninsk, Russia
Email: vzaichick@gmail.com

ABSTRACT

Background: Role of trace elements (TE) in etiology and pathogenesis of Hashimoto's thyroiditis (HT) is unclear. The aim of this exploratory study was to assess whether there were significant changes in thyroid tissue levels of six TE (Br, Cu, Fe Rb, Sr, and Zn) are present in the autoimmune transformed thyroid.

Methods: Six TE of thyroid tissue were determined in 8 patients with HT and 105 healthy populations. The measurements were performed using energy-dispersive X-ray fluorescent analysis.

Results: Elevated mean values of Br and Cu content were found in thyroid with HT in comparison with normal level (in approximately 5.9 and 1.2 times, respectively).

Conclusions: There are considerable changes in some TE contents in tissue of thyroid with HT. Thus, it is reasonable to assume that the levels of these TE in thyroid tissue can be used as HT markers. However, this topic needs additional studies.

KEYWORDS: Hashimoto's thyroiditis; Intact thyroid; Trace elements; Energy-dispersive X-ray fluorescent analysis.

INTRODUCTION

Hashimoto's thyroiditis (HT), also called chronic lymphocytic or autoimmune thyroiditis, is part of the spectrum of chronic autoimmune thyroid diseases [1]. Hashimoto's disease is associated with thyroid autoantibodies production like the most common, thyroid peroxidase and thyroglobulin antibodies, and with lymphocytic inltration [1]. Although the HT was described over 100 years ago the exact mechanism of progressive thyroid tissue destruction as a result of HT is still not sufficiently elucidated. Clinical differentiation between HT, Riedel’s struma and other thyroid benign and malignant nodules is often difficult [2,3]. We hypothesized that disbalance of trace elements (TE) contents in thyroid tissue may play a significant role in etiology and pathogenesis of HT. Furthermore, specific levels of TE contents in autoimmune transformed thyroid tissue may be used as HT biomarkers.

For over 20th century, there was the dominant opinion that all thyroid nodules (TN), including HT, are the elementary consequence of iodine (I) deficiency. However, TN have been found to be a frequent disease even in those countries and regions where the population is never exposed to I deficiency [4]. Moreover, it was shown that iodine excess has severe effects on human health and associated with the development of thyroidal disfunctions and autoimmunity, nodular 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 HT 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].

In addition to I, many other TE are involved in essential physiological functions [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of TE depend on tissue-specific need or tolerance, respectively [13]. Deficiency, overload or an imbalance of the TE may result in cellular dysfunction, 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 employed for the investigation of I and other TE levels in the normal and pathological thyroid gland [16-22]. Level of I in the normal gland was studied in relation to age, gender and some non-thyroidal diseases [23,24]. After that, variations of many other TE content with age in the thyroid of males and females were investigated and age- and gender-dependence of some TE was observed [25-41]. Furthermore, a significant difference between some TE mass fractions in normal and malignant thyroid was demonstrated [42-47].

So far, the etiology and pathogenesis of HT has to be considered as multifactorial. The present study was performed to clarify the role of some TE in the HT etiology. With this in mind, our aim was to assess the bromine (Br), copper (Cu), iron (Fe), rubidium (Rb), strontium (Sr), and zinc (Zn) contents in normal thyroid tissue (NT) and HT affected gland using non-destructive energy dispersive X-ray fluorescent analysis with ^{109}Cd radionuclide application for X-ray fluorescent excitation (^{109}Cd EDXRF). A further aim was to compare the levels of these TE in the NT and HT groups of samples.

MATERIAL AND METHODS

All patients with HT (n=8, 7 females and 1 male, mean age M±SD was 40±10 years, range 34-55) were hospitalized in the Head and Neck Department of the MRRC. Thick-needle puncture biopsy of suspicious lesion of the gland was performed for every persons, to allow morphological examination of affected thyroid tissue and to determine 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 HT.

Normal thyroid 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. Histological examination was used in the NT group to match the age criteria, as well as to confirm the absence of micro-nodules and underlying cancer.

All thyroid samples were divided into two parts using a titanium scalpel [48]. One was used for morphological study while the other was for TE evaluation. All samples for TE analysis were weighed, freeze-dried and homogenized [49]. The pounded sample with mass 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]. Certified Reference Material of the International Atomic Energy Agency CRM IAEA H-4 (animal muscle) 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 thyroid tissue.

Details of the relevant facility for EDXRF, source with ^{109}Cd radionuclide for X-ray fluorescent excitation, 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 NT and HT groups of tissue samples. The difference in the results between two groups (NT and HT) was evaluated by the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

RESULTS

Table 1 presents certain statistical parameters of the Br, Cu, Fe, Rb, Sr, Zn mass fraction in normal thyroid and thyroid with Hashimoto's thyroiditis.

***TABLE 1-** Some statistical parameters of Br, Cu, Fe, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid and thyroid with Hashimoto's thyroiditis*

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal	Br	13.9	12.0	1.3	1.4	54.4	10.0	2.23	50.8
thyroid	Cu	4.23	1.52	0.18	0.50	7.50	4.15	1.57	7.27
n=105	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
Hashimoto's thyroiditis	Br	81.3	38.1	22.0	55.0	125	64.0	55.5	122
n=8	Cu	5.05	0.21	0.15	4.90	5.20	5.05	4.91	5.19
	Fe	165	129	46	94.0	478	112	95.4	423
	Rb	11.4	5.2	1.9	3.80	19.3	11.8	4.36	19.0
	Sr	3.70	2.74	1.03	0.74	6.66	4.81	0.749	6.61
	Zn	97.6	28.0	9.9	50.0	140	97.3	55.1	138

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 of values obtained for Br, Cu, Fe, Rb, Sr, and Zn contents in the NT and HT samples with median of means reported by other researches [9,52-59] depicts in Table 2.

TABLE 2- *Median, minimum and maximum value of means Br, Cu, Fe, Rb, Sr, and Zn contents in normal thyroid and thyroid with Hashimoto's thyroiditis 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
Thyroid	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
Hashimoto's	Br	-	-]	-	81.3±38.1
Thyroiditis	Cu	2.06 (3)	1.66 (31) [9]	4.8±2.8 (14) [59]	5.05±0.21
	Fe	-	-	-	165±129
	Rb	-	-	-	11.4±5.2
	Sr	-	-	-	3.70±2.74
	Zn	54.9 (4)	22.4 (31) [9]	86.4±38.8 (14) [59]	97.6±28.0

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

The ratios of means and the difference between mean values of Br, Cu, Fe, Rb, Sr, Zn mass fractions in normal thyroid and thyroid with Hashimoto's thyroiditis are presented in Table 3.

TABLE 3- *Differences between mean values (M±SEM) of Br, Cu, Fe, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid and thyroid with Hashimoto's thyroiditis*

Element	**Thyroid tissue**				**Ratio**
	Normal thyroid n=105	**Hashimoto's thyroiditis n=8**	**Student's t-test** ***p≤***	**U-test** ***p***	**Hashimoto's thyroiditis to Norm**
Br	13.9±1.3	81.3±22.0	0.091	**≤0.05**	5.85
Cu	4.23±0.18	5.05±0.15	**0.014**	**≤0.01**	1.19
Fe	222±11	165±46	0.262	>0.05	0.74
Rb	9.03±0.66	11.4±1.9	0.258	>0.05	1.26
Sr	4.55±0.37	3.70±1.03	0.464	>0.05	0.81
Zn	112±5	97.6±9.9	0.205	>0.05	0.87

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

DISCUSSION

As was shown before [25,26,51] good agreement of the Br, Cu, Fe, Rb, Sr, and Zn contents analyzed by ^{109}Cd EDXRF with the certified data of CRM IAEA H-4 indicates acceptable accuracy of the results obtained in the study of TE of the thyroid samples presented in Tables 1-3.

The mean values and all selected statistical parameters were calculated for six TE (Br, Cu, Fe, Rb, Sr, and Zn) mass fractions (Table 1). The mass fraction of Br, Cu, Fe, Rb, Sr, and Zn were measured in all, or a major portion of tissue samples in NT and HT groups.

In a general sense values obtained for Br, Cu, Fe, Rb, Sr, and Zn contents in the NT samples (Table 2) agree well with median of mean values reported by other researches [52-58]. A number of values for TE mass fractions in literature were not expressed on a dry mass basis. However, 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.

Data cited in Table 2 for NT 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.

The data on TE levels in thyroid with HT are very limited (Table 2). Results for Cu and Zn obtained in the present study agree well with published data. Information on Br, Fe, Rb, and Sr contents in thyroid with HT was not found in the literature.

The range of means of Br, Cu, Fe, Rb, Sr, and Zn level reported in the literature for NT tissue vary widely (Table 2). 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, mass of the gland, and its functional activity. Not all these factors were strictly controlled in cited studies. However, in our opinion, the main reason for the inter-observer discrepancy can be attributed to the accuracy of the analytical techniques, sample preparation methods, and the inability to take standardized samples from 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 [62,63].

From Table 3, it is observed that in HT samples the mass fraction of Br and Cu are approximately 5.9 and 1.2 times, respectively, higher than in NT. Thus, if we accept the TE contents in the NT group as a norm, we have to conclude that with an autoimmune transformation the Br and Cu level in thyroid tissue significantly changed.

Characteristically, elevated or reduced levels of TE observed in affected tissues are discussed in terms of their potential role in the initiation and promotion of TN. In other words, using the low or high levels of the TE in TN researchers try to determine the role of the deficiency or excess of each TE in the TN etiology. In our opinion, abnormal levels of many TE in TN, including HT, could be and cause, and also effect of thyroid tissue 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. Nevertheless the differences between TE levels in normal and affected thyroid tissue could be used as HT markers.

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 NT and HT. Secondly, the sample size of HT group was relatively small and prevented investigations of TE contents in HT group using differentials like gender, thyroid functional activity, stage of disease, dietary habits of healthy persons and patients with HT. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on autoimmune-specific tissue Br and Cu level alteration and shows the necessity to continue TE research of HT.

CONCLUSION

In this work, TE measurements in tissue samples from NT and HT were performed using ^{109}Cd EDXRF. It was shown that ^{109}Cd EDXRF is an adequate analytical tool for the non-destructive determination of Br, Cu, Fe, Rb, Sr, and Zn content in tissue samples from healthy and affected human thyroid, including needle biopsy samples. It was observed that in HT contents of Br and Cu were significantly higher than in normal tissues. In our opinion, the presented study data strongly suggest that TE plays an important role in thyroid health, as well as in the etiology and pathogenesis of HT. It was assumed that the differences in TE levels in affected thyroid tissue could be used as HT markers.

Declarations

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, former Head of the Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples.

Funding: No funds, grants, or other support was received.

Conflict of interests: The author has no conflicts of interest to declare that are relevant to the content of this article.

Ethical Statement: All studies were approved by the Ethical Committees of the Medical Radiological Research Centre (MRRC), Obninsk. All the procedures performed in studies involving patients 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.

Informed Consent: Not relevant for this paper with no data.

REFERENCES

1. Liontiris MI, Mazokopakis EE (2017) A concise review of Hashimoto thyroiditis (HT) and the importance of iodine, selenium, vitamin D and gluten on the autoimmunity and dietary management of HT patients.Points that need more investigation. Hell J Nucl Med 20(1): 51-56. https://pubmed.ncbi.nlm.nih.gov/28315909/
2. Junik R, Juraniec O, Pypkowski J, Krymer A, Marszałek A (2011) A difficult diagnosis: a case report of combined Riedel's disease and fibrosing Hashimoto's thyroiditis. Endokrynol Pol 62(4): 351-356. https://pubmed.ncbi.nlm.nih.gov/21879477/
3. Heufelder AE, Hay ID (1994) Evidence for autoimmune mechanisms in the evolution of invasive fibrous thyroiditis (Riedel's struma). Clin Investig 72(10): 788-793. https://pubmed.ncbi.nlm.nih.gov/7865983/
4. Derwahl M, Studer H (2000) Multinodular goitre: 'much more to it than simply iodine deficiency'. Baillieres Best Pract Res Clin Endocrinol Metab 14(4): 577-600. https://pubmed.ncbi.nlm.nih.gov/11289736/
5. Zaichick V (1998) Iodine excess and thyroid cancer. J Trace Elem Exp Med 11(4): 508-509.
6. Zaichick V, Iljina T (1998) 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, p. 294-306.
7. Kaličanin D, Brčić L, Ljubetić K, Barić A, Gračan S, Brekalo M, LovrićVT, Kolčić I, Polašek O, Zemunik T, Punda A, Perica VB (2020) Differences in food consumption between patients with Hashimoto's thyroiditis and healthy individuals. Sci Rep 10(1): 10670. https://pubmed.ncbi.nlm.nih.gov/32606353/
8. Vargas-Uricoechea P, Pinzón-Fernández MV, Bastidas-Sánchez BE, 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. https://pubmed.ncbi.nlm.nih.gov/31061736/

9. Stojsavljević A, Rovčanin B, Jagodić J, Drašković-Radojković D, Paunović I, Gavrović-Jankulović M, Manojlović D (2020) Significance of arsenic and lead in Hashimoto's thyroiditis demonstrated on thyroid tissue, blood, and urine samples. Environ Res 186: 109538. https://pubmed.ncbi.nlm.nih.gov/32334172/

10. Fahim YA, Sharaf NE, Hasani IW, et al. (2020) Assessment of thyroid function and oxidative stress state in foundry workers exposed to lead. J Health Pollut 10(27): 200903. https://pubmed.ncbi.nlm.nih.gov/32874759/

11. Liu M, Song J, Jiang Y, et al. (2021) A case-control study on the association of mineral elements exposure and thyroid tumor and goiter. Ecotoxicol Environ Saf 208: 111615. https://pubmed.ncbi.nlm.nih.gov/33396135/

12. Zaichick V (2006) Medical elementology as a new scientific discipline. J Radioanal Nucl Chem 269: 303-309. https://www.deepdyve.com/lp/springer-journals/medical-elementology-as-a-new-scientific-discipline-NYNbKJe7UB

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. BBA Clin 7: 115–119. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5385584/

14. Beyersmann D, Hartwig A (2008) Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 82(8): 493-512. https://pubmed.ncbi.nlm.nih.gov/18496671/

15. Martinez-Zamudio R, Ha HC (2011) Environmental epigenetics in metal exposure. Epigenetics 6(7): 820-827. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3230540/

16. Zaĭchik VE, Raibukhin YuS, Melnik AD, et al. (1970) Neutron-activation analysis in the study of the behavior of iodine in the organism. Med Radiol (Mosk) 15(1): 33-36. https://pubmed.ncbi.nlm.nih.gov/5449249/

17. Zaĭchik VE, Matveenko EG, Vtiurin BM, et al. (1982) Intrathyroid iodine in the diagnosis of thyroid cancer. Vopr Onkol 28(3): 18-24. https://pubmed.ncbi.nlm.nih.gov/7064415/

18. Zaichick V, Tsyb AF, Vtyurin BM (1995) Trace elements and thyroid cancer. Analyst 120(3): 817-821. https://pubmed.ncbi.nlm.nih.gov/7741233/

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(1): 153-161. https://inis.iaea.org/search/search.aspx?orig_q=RN:27073875

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(4): 509-510. https://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=1490208

21. Zaichick V, Zaichick S (1999) Energy-dispersive X-ray fluorescence of iodine in thyroid puncture biopsy specimens. J Trace Microprobe Tech 17(2): 219-232. https://jglobal.jst.go.jp/en/detail?JGLOBAL_ID=200902189182887592

22. Zaichick V (2000) Relevance of, and potentiality for in vivo intrathyroidal iodine determination. Ann N Y Acad Sci 904: 630-632. https://pubmed.ncbi.nlm.nih.gov/10865819/

23. Zaichick V, Zaichick S (1997) Normal human intrathyroidal iodine. Sci Total Environ 206(1): 39-56. https://pubmed.ncbi.nlm.nih.gov/9373990/

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, p.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(1): 31-38. https://scholars.direct/Articles/geriatric-medicine/tghc-1-004.php?jid=geriatric-medicine

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(5): 00028. https://medcraveonline.com/MOJGG/age-related-changes-of-some-trace-element-contents-in-

intact-thyroid-of-males-investigated-by-energy-dispersive-x-ray-fluorescent-analysis.html

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(1): 1002. https://www.jscimedcentral.com/Aging/aging-1-1002.php

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. https://pdfs.semanticscholar.org/bb3f/1dacae61b3a2322d68f02bc5ea9bbce09afa.pdf

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(4): 555644. https://ideas.repec.org/a/adp/jctbeb/v4y2017i4p64-72.html

31. Zaichick V, Zaichick S (2018) Effect of age on chemical element contents in female thyroid investigated by some nuclear analytical methods. MicroMedicine 6(1): 47-61. http://www.journals.tmkarpinski.com/index.php/mmed/article/view/33

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(4): 202-212. https://lupinepublishers.com/biomedical-sciences-journal/fulltext/neutron-activation-and-x-Ray-fluorescent-analysis-in-study-of-association-between-age-and-chemical-element-contents-in-thyroid-of-males.ID.000144.php

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): 1-10. https://www.sciforschenonline.org/journals/bioanalytical-techniques/article-data/JBAS-3-114/JBAS-3-114.pdf

34. Zaichick V, Zaichick S 92018) Association between age and twenty chemical element contents in intact thyroid of males. SM Gerontol Geriatr Res 2(1): 1014. https://www.jsmcentral.org/sm-gerontology/fulltext_smggr-v2-1014.pdf

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(9): 1059–1070. https://link.springer.com/article/10.1007/s40520-018-0906-0?shared-article-renderer

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(3): 107-115. https://www.ecronicon.com/ecgy/pdf/ECGY-07-00198.pdf

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. https://lupinepublishers.com/gynecology-women-health-journal/abstracts/possible-role-of-inadequate-quantities-of-intra-thyroidal-bromine-calcium-and-magnesium.ID.000113.php

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(1): 000108. https://medwinpublishers.com/WHSJ/WHSJ16000108.pdf

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(4): 340-355. https://scientiaricerca.com/srgype/SRGYPE-02-00048.php

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): 1-18. https://www.gudapuris.com/articles/10.31829-2640-6284.crgo2018-2(1)-105.pdf

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(9): 23-37. https://www.actascientific.com/ASMS/pdf/ASMS-02-0144.pdf

42. Zaichick V. Zaichick S (2018) Trace element contents in thyroid cancer investigated by energy dispersive X-ray fluorescent analysis. American Journal of Cancer Research and Reviews 2: 5.https://escipub.com/ajocr-2017-12-2801/

43. Zaichick V., Zaichick S (2018) Trace element contents in thyroid cancer investigated by instrumental neutron activation analysis. J Oncol Res 2(1): 1-13. http://gudapuris.com/articles/10.31829-2637-6148.jor2018-2(1)-103.pdf

44. Zaichick V, Zaichick S (2018) Variation in selected chemical element contents associated with malignant tumors of human thyroid gland. Cancer Studies 2(1): 2 https://www.researchgate.net/publication/341612609_Variation_in_Selected_Chemical_Element_Contents_Associated_with_Malignant_Tumors_of_Human_Thyroid_Gland

45. Zaichick V, Zaichick S (2018) Twenty chemical element contents in normal and cancerous thyroid. Int J Hematol Blo Dis 3(2): 1-13. https://symbiosisonlinepublishing.com/hematology/hematology21.php

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. https://jcmtjournal.com/article/view/2938

47. Zaichick V, Zaichick S (2018) Fifty trace element contents in normal and cancerous thyroid. Acta Scientific Cancer Biology 2(8): 21-38. https://www.actascientific.com/ASCB/pdf/ASCB-02-0061.pdf

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(12): 1200-1205. https://inis.iaea.org/search/search.aspx?orig_q=RN:28056394

49. Zaichick V, Zaichick S (1997) A search for losses of chemical elements during freeze-drying of biological materials. J Radioanal Nucl Chem 218(2): 249-253. https://link.springer.com/article/10.1007/BF02039345

50. Zaichick V (1995) Applications of synthetic reference materials in the medical Radiological Research Centre. Fresenius J Anal Chem 352: 219-223. https://link.springer.com/article/10.1007/BF00322330

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(6): 464-469. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/xrs.1370

52. Zhu H, Wang N, Zhang Y, et al. (2010) Element contents in organs and tissues of Chinese adult men. Health Phys 98(1): 61-73. https://pubmed.ncbi.nlm.nih.gov/19959952/

53. Salimi J, Moosavi K, Vatankhah S, et al. (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(4): 211-216. http://ijrr.com/browse.php?a_id=32&sid=1&slc_lang=en

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, 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(3-4): 333-339. https://www.researchgate.net/publication/223853105_Trace_elemental_analysis_of_adenoma_and_carcinoma_thyroid_by_PIXE_method

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(2): 98-102. https://pubmed.ncbi.nlm.nih.gov/5807109/

57. Boulyga SF, Zhuk IV, Lomonosova EM, et al. (1997) Determination of microelements in thyroids of the inhabitants of Belarus by neutron activation analysis using the k_0-method. J Radioanal Nucl Chem 222 (1-2): 11-14. https://link.springer.com/article/10.1007/BF02034

238

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. Predtechenskaya VC (1975) Nucleic acids and trace elements in thyroid pathology. Proceedings of the Voronezh Medical Faculty 94: 85-87.

60. Katoh Y, Sato T, Yamamoto Y (2002) Determination of multielement concentrations in normal human organs from the Japanese. Biol Trace Elem Res 90(1-3): 57-70. https://pubmed.ncbi.nlm.nih.gov/12666826/

61. Schroeder HA, Tipton IH, Nason AP (1972) Trace metals in man: strontium and barium. J Chron Dis 25(9): 491-517. https://pubmed.ncbi.nlm.nih.gov/4647214/

62. 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. https://inis.iaea.org/search/search.aspx?orig_q=RN:29019688

63. Zaichick V (2004) Losses of chemical elements in biological samples under the dry aching process. Trace Elements in Medicine 5(3):17–22. https://www.researchgate.net/publication/284107719_Losses_of_chemical_elements_in_biological_samples_under_the_dry_aching_process