Colloid nodular goiter (CNG) is the most common disease of the thyroid, even in non-endemic regions, but the etiology of CNG is unclear. It is known that not merely iodine (I) but other chemical elements (Che) are involved in goitrogenesis. The current study was performed to clarify the preferential accumulation of some Che either in the colloid or in cells of the thyroid gland.

Methods: Eight Che: Bromine, calcium, chlorine (Cl), I, potassium, magnesium, and sodium (Na) in the thyroid tissues with diagnosed CNG were prospectively evaluated in 16 patients with macrofolicular CNG and 13 patients with microfolicular CNG. The control group included thyroid tissue samples from 105 healthy individuals. Measurements were conducted using non-destructive instrumental neutron activation analysis with high-resolution spectrometry of short-lived radionuclides.

Results: It was found that in macrofolicular CNG, the mass fraction of Cl and Na was 2.57 and 1.82 times, respectively, higher than in tissues of the normal thyroid. In microfolicular CNG, the mass fraction of I was 59% lower, whereas the mass fraction of Na was 67% higher than in tissues of the normal thyroid. The level of I in macrofolicular goiter was 2.08 times higher than in microfolicular goiter.

Conclusion: There are substantial changes in Che contents in the goitrous transformed tissue of the thyroid, which depend on the histology of the goiter.

Keywords: Macro- and micro-follicular colloid nodular goiter of thyroid, intact thyroid, chemical elements, instrumental neutron activation analysis.

INTRODUCTION

Colloid nodular goiter (CNG) is the most common thyroid disease, even in non-endemic regions [1]. CNG is clinically identified in about 4% of people older than 30 years [1]. CNG is a benign lesion; however, during clinical examination, it can imitate malignant tumors. Furthermore, the origination of CNG can stipulate the beginning of the malignant transformation of the thyroid gland [2]. Up to now, the etiology of CNG is unclear, and it is probably multifactorial [3]. There is an opinion that CNG occurs when the thyroid is not able to meet the metabolic demands of the body with adequate hormone production. The thyroid gland compensates by enlarging, which usually overcomes mild deficiencies of thyroid hormones. For over the 20th century, there was the governing opinion that NG is the straightforward sequel of iodine (I) deficiency. Although, it was found that NG is a frequent disease even in those countries and regions where the inhabitants are never exposed to I shortage [4]. Moreover, it was found that I excess has severe effects on human health and is associated with the presence of thyroid dysfunctions and autoimmunity, NG and diffuse goiter, benign and malignant tumors of the gland [5-8]. It was also demonstrated that besides the I deficiency and excess, many other dietary, environmental, and occupational factors are associated with the NG incidence [9-11]. Among them, a disruption of evolutionary stable input of many chemical elements (Che) in the human body after the industrial revolution plays a significant role in the etiology of thyroidal disorders [12].

In our earlier studies, the complex of in vivo and in vitro nuclear analytical and related methods was developed and used for the investigation of I and other Che contents in the normal and pathological thyroid [16-22]. I level in the normal thyroid was scrutinized in relation to age, gender, and some non-thyroidal diseases [23,24]. Hereafter, variations of Che content with age in the thyroid of males and females were studied, and age and gender dependence of some Che was perceived [25-41]. In addition, a significant difference between some Che 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 clear that these two types of CNG have different volume ratios, "colloid to cells."

The present study was executed to elucidate the preferential accumulation of some Che either in the colloid or in cells of the thyroid gland. Having this in mind, we focused on assessing the bromine (Br), calcium (Ca), chlorine (Cl), I, potassium (K), magnesium (Mg), manganese (Mn), and sodium (Na) contents in macro- and micro-follicular CNG tissue using non-destructive instrumental neutron activation analysis with high-resolution spectrometry of short-lived radionuclides (INAA-SLR). A further objective was to compare the levels of these Che 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 Che 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.
Table 1: Some statistical parameters of Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in normal thyroid and colloid nodular goiter of different histology (macro- and micro-follicular)

| Tissue  | Element | Mean  | SD    | SEM   | Min   | Max   | Median | P 0.025 | P 0.975 |
|---------|---------|-------|-------|-------|-------|-------|--------|---------|---------|
| Normal  | Br      | 16.3  | 11.6  | 1.3   | 1.90  | 66.9  | 13.6   | 2.57    | 51.0    |
|         | Ca      | 1692  | 1022  | 109   | 4164  | 6120  | 1451   | 460     | 3905    |
|         | Cl      | 3400  | 1452  | 174   | 4130  | 6000  | 3470   | 12.44   | 5869    |
|         | I       | 1841  | 1027  | 107   | 114   | 5061  | 1695   | 230     | 4232    |
|         | K       | 6071  | 2773  | 306   | 1740  | 14300 | 5477   | 2541    | 13285   |
|         | Mg      | 215   | 139   | 16.5  | 660   | 930   | 271    | 81.6    | 541     |
|         | Mn      | 1.35  | 0.58  | 0.07  | 0.510 | 4.18  | 1.32   | 0.537   | 2.23    |
|         | Na      | 6702  | 1764  | 178   | 3050  | 13453 | 6690   | 3855    | 10709   |
| Macro   | Br      | 422   | 233   | 10.4  | 12.0  | 65.3  | 40.3   | 13.6    | 65.3    |
| n=16    | Ca      | 1455  | 999   | 258   | 209   | 4333  | 1264   | 278     | 3632    |
|         | Cl      | 8749  | 4089  | 1546  | 4226  | 16786 | 8191   | 4487    | 15880   |
|         | I       | 1587  | 1087  | 302   | 300   | 3715  | 1206   | 322     | 3686    |
|         | K       | 6254  | 1801  | 465   | 3801  | 9936  | 6185   | 3917    | 9641    |
|         | Mg      | 345   | 158   | 41    | 13.0  | 531   | 374    | 30.5    | 531     |
|         | Mn      | 1.35  | 0.68  | 0.18  | 0.370 | 2.70  | 1.20   | 0.432   | 2.63    |
|         | Na      | 12.211| 4164  | 1075  | 7229  | 22381 | 11.056 | 7326    | 20493   |
| Micro   | Br      | 19.4  | 7.1   | 3.5   | 13.7  | 29.6  | 17.1   | 13.9    | 28.7    |
| n=13    | Ca      | 1152  | 610   | 249   | 288   | 2101  | 1092   | 358     | 2025    |
|         | Cl      | 9977  | 3939  | 2274  | 5462  | 12712 | 11756  | 5777    | 12664   |
|         | I       | 762   | 600   | 173   | 141   | 1936  | 586    | 173     | 1929    |
|         | K       | 6932  | 2783  | 1052  | 3353  | 10318 | 6461   | 3423    | 10193   |
|         | Mg      | 328   | 134   | 51    | 122   | 497   | 371    | 134     | 486     |
|         | Mn      | 2.40  | 1.70  | 0.69  | 0.450 | 5.50  | 1.93   | 0.619   | 5.16    |
|         | Na      | 11167 | 2472  | 1009  | 8065  | 14584 | 11518  | 8153    | 14239   |

M: Arithmetic mean, SD: Standard deviation, SEM: Standard error of the 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: Differences between mean values (M±SEM) of Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in normal thyroid and macrofollicular colloid nodular goiter

| Element | Normal thyroid n=105 | Macrofollicular goiter n=16 | U-test p | Student's t-test p≤ | Ratio | Goiter to norm |
|---------|----------------------|-----------------------------|----------|-------------------|------|---------------|
| Br      | 16.3±1.3             | 42.2±10.4                   | 0.067    | >0.05             | 2.59 |               |
| Ca      | 169±2.19             | 1455±258                    | 0.371    | >0.05             | 0.86 |               |
| Cl      | 340±174              | 8749±1546                   | 0.013    | ≤0.01             | 2.57 |               |
| I       | 181±1.07             | 1587±302                    | 0.439    | >0.05             | 0.86 |               |
| K       | 607±306              | 625±465                     | 0.745    | >0.05             | 1.03 |               |
| Mg      | 285±17               | 345±41                      | 0.194    | ≤0.05             | 1.21 |               |
| Mn      | 1.35±0.07            | 1.35±0.18                   | 0.966    | >0.05             | 1.00 |               |
| Na      | 6702±1785            | 12,211±1075                 | 0.00015  | ≤0.01             | 1.82 |               |

M: Arithmetic mean, SEM: Standard error of mean, significant values are in bold

Table 3: Differences between mean values (M±SEM) of Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in normal thyroid and microfollicular colloid nodular goiter

| Element | Normal thyroid n=105 | Microfollicular goiter n=13 | U-test p | Student's t-test p≤ | Ratio | Goiter to norm |
|---------|----------------------|----------------------------|----------|-------------------|------|---------------|
| Br      | 16.3±1.3             | 19.4±3.5                   | 0.464    | >0.05             | 1.19 |               |
| Ca      | 169±2.19             | 1152±49                    | 0.078    | >0.05             | 0.68 |               |
| Cl      | 340±174              | 9977±2274                  | 0.101    | ≤0.05             | 2.93 |               |
| I       | 181±1.07             | 762±173                    | 0.00003  | ≤0.01             | 0.41 |               |
| K       | 607±306              | 6932±1052                  | 0.458    | >0.05             | 1.14 |               |
| Mg      | 285±17               | 328±51                     | 0.436    | >0.05             | 1.15 |               |
| Mn      | 1.35±0.07            | 2.40±0.69                  | 0.192    | >0.05             | 1.78 |               |
| Na      | 6702±1785            | 11,167±1009                | 0.0063   | ≤0.01             | 1.67 |               |

M: Arithmetic mean, SEM: Standard error of the mean, significant values are in bold

Table 4: Differences between mean values (M±SEM) of Br, Ca, Cl, I, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in macro- and micro-follicular colloid nodular goiter

| Element | Normal thyroid n=105 | Macrofollicular goiter n=16 | Microfollicular goiter n=13 | U-test p | Student's t-test p≤ | Ratio | Macro to Micro |
|---------|----------------------|-----------------------------|----------------------------|----------|-------------------|------|---------------|
| Br      | 42.2±10.4            | 19.4±3.5                   | 0.094                       | >0.05    | 2.18              |      |               |
| Ca      | 1455±258             | 1152±49                    | 0.411                       | >0.05    | 1.26              |      |               |
| Cl      | 8749±1546            | 9977±2274                  | 0.678                       | >0.05    | 0.88              |      |               |
| I       | 1587±302             | 762±173                    | 0.028                       | ≤0.01    | 2.08              |      |               |
| K       | 625±465              | 6932±1052                  | 0.571                       | >0.05    | 0.90              |      |               |
| Mg      | 345±41               | 328±51                     | 0.901                       | >0.05    | 1.05              |      |               |
| Mn      | 1.35±0.18            | 2.40±0.69                  | 0.194                       | >0.05    | 0.56              |      |               |
| Na      | 12,211±1075          | 11,167±1009                | 0.489                       | >0.05    | 1.09              |      |               |

M: Arithmetic mean, SEM: Standard error of mean, significant values are in bold

in tissues of the normal thyroid. From Table 3, it is observed that in microfollicular CNG, the mass fraction of I is 59% lower, whereas the mass fraction of Na is 67% higher than in tissues of the normal thyroid. Thus, if we accept the ChE contents in thyroid glands in the control group as a norm, we have to conclude that the Cl, I, and Na level in thyroid tissue can be notably changed with a goitrous transformation.

Association between ChE levels and relative volume of colloid and cells

Comparison mass fraction of Br, Ca, Cl, I, K, Mg, Mn, and Na in macro- and micro-follicular CNG shown that level of I in macrofollicular goiter is 2.08 times higher than in microfollicular goiter (Table 4). Because the relative volume of colloid in the macrofollicular CNG is higher than in the microfollicular CNG, it is possible to conclude that I increasingly associated with colloid.

Comparison with published data

The published data on ChE contents in the CNG compared to normal levels are very sparse and contradictory. For example, information about Cl content in CNG was not found. Merely, one paper with results on Na level in normal thyroid and CNG was published in 1963 by Kamenev [52], but changes of this electrolyte level in goitrous thyroid were not shown. A relative good agreement there is only for I, since most of the published studies showed a significant decrease of I content in the CNG [53-56]. Information on the ChE contents in macro- or micro-follicular CNG, also about the association between ChE level and relative volume of colloid and cells in goitrous tissue, was not found.

Limitations

This study has some limitations. First, analytical techniques used in this study measure merely eight ChE (Br, Ca, Cl, I, K, Mg, Mn, and Na) mass fractions. Future studies should be aimed toward using other analytical methods which will elongate the list of ChE investigated in normal and goitrous thyroid. Second, the sample size of macro- or micro-follicular CNG groups was relatively small and averted investigations of ChE contents in CNG group using differentials such as gender, stage of disease, and dietary habits of healthy persons and patients with CNG. Finally, the generalization of our outcomes may be bounded...
to the Russian population. Despite these constraints, this study provides evidence on goiter-specific tissue CGl, I, and Na level alterations demonstrates associations between I and relative volume of colloid in CNG, and shows the necessity to continue Čhe research of CNG of different histology.

CONCLUSION

In this work, Čhe analysis was carried out in the tissue samples of normal and goitrous thyroid using INAA-SLR. It was shown that INAA-SLR is an adequate analytical tool for the non-destructive determination of Br, Ca, Cl, I, K, Mg, Mn, and Na content in the tissue samples of human thyroid in norm and pathology, including needle biopsy cores. It was perceived the considerable changes in Čhe contents in the goitrous transformed tissue of thyroid, which depends on the histology of goiter. It was found that I predominately accumulates in colloids of CNG.

ACKNOWLEDGMENTS

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

REFERENCES

1. Stuchi LP, Castanhole-Nunes MM, Maniezzo-Stuchi N, Biselli-Chicote P, Henrique T, Neto JA, et al. EGFA and NFE2L2 gene expression and regulation by microRNAs in thyroid papillary cancer and colloids. Environ Sci Pollut Res 2020;11:954.
2. Campbell MJ, Seib CD, Candell L, Gosnell JE, Duh QY, Clark OH, et al. 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. Scand J Surg 2004;93:278-81.
4. Derwahl M, Studer H. Multinodular goitre: Much more to it than simply iodine deficiency. Baillieres Best Pract Res Clin Endocrinol Metab 2000;14:577-600.
5. Zaichick V. Iodine excess and thyroid cancer. J Trace Elem Exp Med 1998;11:508-9.
6. Zaichick V, Ilijina T. Dietary iodine supplementation effect on the rat thyroid 131I blastomogenic action. In: Die Bedeutung der Mengen- und Spurenelemente. 18. Arbeitstagung. Jena: Friedrich-Schiller-Universität; 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;11:2757.
8. Vargas-Urcochea P, Pinzón-Fernández MV, Bastidas-Sánchez BE, Jojoo-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. Stojasavljević A, Rovčanin K, Krstić D, Borković-Minić S, Paunović I, Đikić A, et al. 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;12:255-64.
10. Fahim YA, Sharaf NE, Hasani IW, Ragab EA, Abdelhamik HK. Assessment of thyroid function and oxidative stress state in foundry workers exposed to lead. J Health Pollut 2020;10:200093.
11. Liu M, Song J, Jiang Y, Lin Y, Peng J, Liang H, et al. 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;269:303-9.
13. Moncayo R, Moncayo H. A post-publication analysis of the idealized upper reference value of 2.5 μIU/L for TSH: Time to support the thyroid axis with magnesium and iron especially in the setting of reproduction medicine. BBA Clin 2017;7:115-9.
14. Beyersmann D, Hadwig A. Calcium and magnesium compounds: Recent insight into molecular and cellular mechanisms. Arch Toxicol 2008;82:493-512.
15. Martínez-Zamudio R, Ha HC. Environmental epigenetics in metal exposure. Epigenetics 2011;6:820-7.
16. Zaichick VE, RaibakhinYuS, Melnik AD, Cherkashin VI. Neutron activation analysis of the behavior of iodine in the organism.
coupled plasma mass spectrometry. Acta Sci Med Sci 2018;2:23-37.
42. Zaichick V, Zaichick S. Trace element contents in thyroid cancer investigated by energy dispersive X-ray fluorescent analysis. Am J Cancer Res Rev 2018;2:5.
43. Zaichick V, Zaichick S. Trace element contents in thyroid cancer investigated by instrumental neutron activation analysis. J Oncol Res 2018;2:1-13.
44. Zaichick V, Zaichick S. Variation in selected chemical element contents associated with malignant tumors of human thyroid gland. Cancer Stud 2018;2:2.
45. Zaichick V, Zaichick S. Twenty chemical element contents in normal and cancerous thyroid. Int J Hematol Blood 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 Sci Cancer Biol 2018;2:21-38.
48. Zaichick V, Zaichick S. Instrumental effect on the contamination of biomedical samples in the course of sampling. J Anal Chem 1996;51:1200-5.
49. Zaichick V, Zaichick S. A search for losses of chemical elements during freeze-drying of biological materials. J Radioanal Nucl Chem 1997;218:249-53.
50. Zaichick V. Applications of synthetic reference materials in the medical radiological research centre. Fresenius J Anal Chem 1995;352:219-23.
51. Korelo AM, Zaichick V. Software to optimize the multielement INAA of medical and environmental samples. In: Activation Analysis in Environment Protection. Dubna, Russia: Joint Institute for Nuclear Research;1993. p. 326-32.
52. Kamenev VF. About trace element contents in thyroid of adults. In: Trace Elements in Agriculture and Medicine. Ulan-Ude: Buryatia Publishing-House; 1963. p. 12-6.
53. Le Blank AD, Bell RL, Johnson PhC. Measurement of 127I concentration in thyroid tissue by X-ray fluorescence. J Nucl Med 1973;14:816-9.
54. Kohler H, Studer H. Biochemical changes in “warm” and “cold” thyroid nodules. Therapiewoche 1981;31:1539-45.
55. Tadros TG, Maisey MN, Ng Tang Fui SC, Turner P. The iodine concentration in benign and malignant thyroid nodules measured by X-ray fluorescence. Br J Radiol 1981;54:626-9.
56. Błazewicz A, Dolliver W, Sivsammy S, Deol A, Randhawa R, Orlicz-Szczesna G, et al. 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;878:34-8.