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# Trace Element Contents in Thyroid Cancer Investigated by Energy Dispersive X-Ray Fluorescent Analysis

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## ABSTRACT

**Background:** Thyroid cancer is an internationally important health problem. The aim of this exploratory study was to evaluate whether significant changes in the thyroid tissue levels of Br, Cu, Fe, Rb, Sr, and Zn exist in the malignantly transformed thyroid.
**Methods:** Thyroid tissue levels of six trace elements (Br, Cu, Fe, Rb, Sr, and Zn) were prospectively evaluated in 41 patients with thyroid malignant tumors and 105 healthy inhabitants. Measurements were performed using 109Cd 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 trace element analysis.
**Results:** It was found that contents of Br, Cu, and Rb were significantly higher (approximately 10, 3.4, and 1.4 times, respectively) and content of Zn were slightly, but significantly, lower (25%) in cancerous tissues than in normal tissues.
**Conclusions:** There are considerable changes in trace element contents in the malignantly transformed tissue of thyroid.

**Keywords:** Thyroid malignant tumors - Intact thyroid - Trace elements - Energy-dispersive X-ray fluorescent analysis

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## Introduction

Thyroid cancer (TC) is the most common endocrine malignancy. TC incidence has dramatically increased in the recent decades [1]. During the same period no other cancer has increased as much as TC. With the worldwide increase in the incidence of TC, it has become the fifth most common cancer in women [2-4]. In some countries, the incidence of TC has increased extremely fast, and it has been the most common cancer for the last years [5].

Although the etiology of TC is unknown, several risk factors including deficiency or excess of such micronutrient as iodine (I) have been well identified [6-17]. It was also reported that incidence of TC and mortality from this disease increases progressively with advancing age {18,19]. For example, a 37-fold increase in hazard ratio from age <40 years to age >70 years was showed in the study of 3664 TC patients that received surgery and adjuvant treatment at Memorial Sloan Kettering Cancer Center from the years 1985 to 2010 [19].

Besides I involved in thyroid function, other trace elements 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 (mutagenic, carcinogenic) properties of trace elements depend on tissue-specific need or tolerance, respectively [20]. Excessive accumulation or an imbalance of the trace elements may disturb the cell functions and may result in cellular degeneration, death or malignant transformation [20-22].

In our previous study a significant positive correlation between age and some trace element contents in the thyroid was observed [23-28]. For example, a strongly pronounced tendency of age-related increase in bromine (Br), coper (Cu), rubidium (Rb) and zinc (Zn) mass fraction was demonstrated by us using $^{109}$Cd radionuclide-induced energy dispersive X-ray fluorescent (EDXRF) analysis [26,28]. In addition, a significant positive correlation was seen between the contents of Zn and Rb in female thyroid [28], as well as between Zn and Cu, Zn and iron (Fe), and also Zn and strontium (Sr) in male thyroid [26]. It was concluded that high intra-thyroidal Zn concentrations are probably one of the main factors acting in both initiation and promotion stages of thyroid carcinogenesis [26,28], as it was earlier shown by us for a prostate gland [29-34]. Moreover, it seems fair to suppose that besides Zn, such trace elements as Br, Cu, Fe, Rb, and Sr also play a role in the pathophysiology of the thyroid.

This work had two aims. The first was to assess the Br, Cu, Fe, Rb, Sr, and Zn contents in TC tissue using EDXRF analysis. The second aim was to compare the levels of trace elements in the malignant 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, Obninsk.

## Material and Methods

All patients suffered from TC (n=41, mean age M±SD was 46±15 years, range 16-75) 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 trace element contents. In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. Histological conclusions for malignant tumors were: 25 papillary adenocarcinomas, 8 follicular adenocarcinomas, 7 solid carcinomas, and 1 reticulosarcoma.

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 [35]. One was used for morphological study while the other was intended for trace element analysis. After the samples intended for trace element analysis were weighed, they were freeze-dried and homogenized [36]. 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 trace elements by comparison with known data for standard, aliquots of commercial, chemically pure compounds and synthetic reference materials were used [37]. 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 results of quality control were presented in our earlier publications concerning the EDXRF analysis of human thyroid and prostate tissue [26,28,38-40].

All thyroid samples were prepared in duplicate, and mean values of trace element 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 trace element contents in normal and cancerous thyroid tissue. The difference in the results between two groups (normal thyroid and TC) 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 trace elements 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 cancerous thyroid tissue.

The comparison of our results with published data for Br, Cu, Fe, Rb, Sr, and Zn mass fraction in normal and cancerous thyroid [41-52] 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 cancerous 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 trace elements of the thyroid samples presented in Tables 2–4.

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 cancerous tissue samples.

**Comparison with published data:** Values obtained for Br, Cu, Fe, Rb, Sr, and Zn contents in the normal human thyroid (Table 3) agree well with median of mean values reported by other researches [41-52]. Data cited in Table 3 also includes samples obtained from patients who died from different non-endocrine diseases. A number of values for trace element mass fractions were not

**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.

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

| 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 |
| n=105 | 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 |
| Cancer | Br | 139 | 203 | 36 | 6.2 | 802 | 50.2 | 7.75 | 802 |
| n=41 | Cu | 14.5 | 9.4 | 2.6 | 4.00 | 32.6 | 10.9 | 4.21 | 31.4 |
| | Fe | 238 | 184 | 30 | 54 | 893 | 176 | 55.0 | 680 |
| | Rb | 12.4 | 5.00 | 0.79 | 4.80 | 27.4 | 11.5 | 4.90 | 20.0 |
| | Sr | 6.25 | 7.83 | 1.63 | 0.93 | 30.8 | 3.00 | 0.985 | 25.0 |
| | Zn | 84.3 | 57.4 | 9.2 | 36.7 | 277 | 65.3 | 39.0 | 273 |

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 3:** Median, minimum and maximum value of means Br, Cu, Fe, Rb, Sr, and Zn contents in normal and cancerous 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) [41] | 284±44 (14) [42] | 13.9±12.0 |
| | Cu | 6.1 (57) | 1.42 (120) [43] | 220±22 (10) [44] | 4.23±1.52 |
| | Fe | 252 (21) | 56 (120) [43] | 2444±700 (14) [42] | 222±102 |
| | Rb | 12.3 (9) | ≤0.85 (29) [45] | 294±191 (14) [42] | 9.03±6.17 |
| | Sr | 0.73 (9) | 0.55±0.26 (21) [46] | 46.8±4.8 (4) [44] | 4.55±3.22 |
| | Zn | 118 (51) | 32 (120) [43] | 820±204 (14) [42] | 112±44 |
| Cancer | Br | 15.7 (4) | 9.6 (1) [47] | 160±112 (3) [42] | 139±203 |
| | Cu | 6.8 (11) | 4.7±1.8 (22) [48] | 51.6±5.2 (4) [44] | 14.5±9.4 |
| | Fe | 316 (8) | 69±51 (3) [49] | 5588±556 (4) [44] | 238±184 |
| | Rb | 14.7 (2) | 11,5 (10) [50] | 17.8±9.7 (5) [50] | 12.4±5.0 |
| | Sr | - | - | - | 6.25±7.83 |
| | Zn | 112 (13) | 48±8 (5) [51] | 494±37 (2) [52] | 84.3±57.4 |

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

**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 cancerous thyroid

| Element | Thyroid tissue | | | | Ratio |
|---|---|---|---|---|---|
| | Norm n=105 | Cancer n=41 | Student's t-test *p*≤ | U-test *p* | Cancer to Norm |
| Br | 13.9±1.3 | 139±36 | **0.0015** | **≤0.01** | 10.0 |
| Cu | 4.23±0.18 | 14.5±2.6 | **0.0019** | **≤0.01** | 3.43 |
| Fe | 222±11 | 238±30 | 0.610 | >0.05 | 1.07 |
| Rb | 9.03±0.66 | 12.4±0.79 | **0.0013** | **≤0.01** | 1.37 |
| Sr | 4.55±0.37 | 6.25±1.63 | 0.319 | >0.05 | 1.37 |
| Zn | 112±5 | 84.3±9.2 | **0.0086** | **≤0.01** | 0.75 |

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

expressed on a dry mass basis by the authors of the cited references. However, we calculated these values using published data for water (75%) [53] and ash (4.16% on dry mass basis) [54] contents in thyroid of adults.

The range of means of Br, Cu, Fe, Rb, Sr, and Zn level reported in the literature for normal and for untreated cancerous thyroid vary widely (Table 3). This can be explained by a dependence of trace element content on many factors, including the region of the thyroid, from which the sample was taken, age, gender, ethnicity, mass of the gland, and the cancer stage. Not all these factors were strictly controlled in cited studies. Another and, in our opinion, leading cause 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 reported papers 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 by use of these methods some quantities of certain trace elements are lost as a result of this treatment That concern not only such volatile halogen as Br, but also other trace elements investigated in the study [55-57].

In cancerous tissues (Table 3) our results were comparable with published data for Cu, Fe, Rb, and Zn contents. The obtained mean for Br was almost one order of magnitude higher median of previously reported means but inside the range of means (Table 3). No published data referring Sr contents of cancerous thyroid tissue were found.

**Effect of malignant transformation on chemical element contents:** From Table 4, it is observed that in cancerous tissue the mass fraction of Zn is 25% lower whereas mass fractions of Br, Cu, and Rb are 10, 3.4, and 1.4 times, respectively, higher than in normal tissues of the thyroid. Thus, if we accept the trace element contents in thyroid glands in the control group as a norm, we have to conclude that with a malignant transformation the Br, Cu, Rb, and Zn in thyroid tissue significantly changed.

**Role of trace elements in malignant transformation of the thyroid:** Characteristically, elevated or reduced levels of trace elements observed in cancerous tissues are discussed in terms of their potential role in the initiation and promotion of thyroid cancer. In other words, using the low or high levels of the trace element in cancerous tissues researchers try to determine the carcinogenic role of the deficiency or excess of each trace element in investigated organ. In our opinion, abnormal levels of many trace elements in tumor could be and cause, and also effect of malignant transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in trace element level in pathologically altered tissue is the reason for alterations or vice versa.

*Bromine.* The Br is one of the most abundant and ubiquitous of the recognized trace elements 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 [58].

In our previous studies it was found a significant age-related increase of Br content in human thyroid [23,26-28]. Therefore, a goitrogenic and, probably, carcinogenic effect of excessive Br level in the thyroid of old females was assumed. On the one hand, elevated level of Br in TC tissues, observed in the present study, supports this conclusion. But, on the other hand, bromide compounds, especially potassium bromide (KBr), sodium bromide (NaBr), and ammonium bromide

($NH_4Br$), are frequently used as sedatives in Russia [59]. It may be the reason for elevated levels of Br in specimens of patients with TC. Nevertheless, the accumulation of Br in neoplastic thyroids could possibly be explored for diagnosis of TC.

*Coper.* Cu 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 [60-62]. Thus, Cu accumulation in thyroid parenchyma with age may be involved in oxidative stress, dwindling gland function, and increasing risk of goiter or cancer [26,28]. The significantly elevated level of Cu in thyroid malignant tumors, 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 thyroid malignant tumors etiology and metabolism. Anyway, the accumulation of Cu in neoplastic thyroids could possibly be explored for diagnosis of TC.

*Rubidium*. As for Rb, there is very little information about its effects in organisms. No negative environmental effects have been reported. Rb is only slightly toxic on an acute toxicological basis and would pose an acute health hazard only when ingested in large quantities [63]. Rb has some function in immune responce [64], probably by supporting cell differentiation [65]. Potassium (K) and Rb are in the first group of the periodic table. Rb, like K, seems to be concentrated in the intracellular space and transfered through membrane by the Na+K+-ATPase pump. An overload of Rb could modulate proliferative responses of the cell, as was shown for bone marrow leukocytes [19]. In our previous studies it was found a significant age-related increase of Rb content in female thyroid [23,26-28]. Therefore, a goitrogenic and, probably, carcinogenic effect of excessive Rb level in the thyroid of old females was assumed. Elevated level of Rb in TC tissues, observed in the present study, supports this conclusion. Anyway, the accumulation of Rb in malignant thyroid tumors could possibly be explored for diagnosis of TC.

*Zinc.* Zn is active in more than 300 proteins and over 100 DNA-binding proteins, including the tumor suppressor protein p53, a Zn-binding transcription factor acting as a key regulator of cell growth and survival upon various forms of cellular stress. p53 is mutated in half of human tumors and its activity is tightly regulated by metals and redox mechanisms. On the other hand, excessive intracellular Zn concentrations may be harmful to normal metabolism of cells [66]. By now much data has been obtained related both to the direct and indirect action of intracellular Zn on the DNA polymeric organisation, replication and lesions, and to its vital role for cell division [67-69]. Moreover, it is known that Zn is an inhibitor of the Ca-dependent apoptotic endonuclease, which takes part in the internucleosomal fragmentation of DNA. Consequently there is a reduction of cell apoptosis [70]. Other actions of Zn have been described. They include its action as a potent anti-apoptotic agent [71-74]. All these facts allowed us to speculate that age-related overload Zn content in female thyroid, as was found in our previous study [25,28], is probably one of the factors in etiology of thyroid goiter and malignant tumors. Therefore, the reduced Zn content in thyroid malignant tumors in comparison with normal level, detected in this study, is still to be cleared.

Our findings show that mass fraction of Ba, Cu, Rb and Zn are significantly different in TC as compared to normal thyroid tissues (Tables 4). Thus, it is plausible to assume that levels of these trace elements in thyroid tissue can be used as tumor markers. However, this subjects needs in additional studies.

**Limitations:** This study has several limitations. Firstly, analytical techniques employed in this study measure only six trace element (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 chemical elements investigated in normal and cancerous thyroid tissue. Secondly, the sample size of TC group was relatively small. It was not allow us to carry out the investigations of trace element contents in TC group using differentials like gender, histological types of tumors, stage of disease, and dietary habits of healthy persons and patients with TC. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on cancer-specific tissue Br, Cu, Rb, and Zn level alteration and shows the necessity the need to continue trace element research of malignant thyroid tumors.

## Conclusion

In this work, trace elemental analysis was carried out in the tissue samples of normal thyroid and malignant tumors of 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, including needle-biopsy cores. It was observed that in cancerous tissues content of Zn was significantly lower and contents of Br, Cu and Rb were significantly higher than in normal tissues. In our opinion, the abnormal decrease in level of Zn, as well as the increase in levels of Br, Cu, and Rb in cancerous tissue might demonstrate an involvement of these elements in etiology and pathogenesis of malignant thyroid tumors. It was supposed that elevated levels of Br, Cu, and Rb, as well as reduced level of Zn in thyroid tissue can be used as tumor markers.

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## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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