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Research Article

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Content of Copper, Iron, Iodine, Rubidium, Strontium and Zinc in Thyroid Malignant Nodules and Thyroid Tissue adjacent to Nodules

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Abstract

Thyroid malignant nodules (TMNs) are the most common endocrine cancer. The etiology and pathogenesis of TMNs must be considered as multifactorial. Diagnostic evaluation of TMNs represents a challenge, since there are numerous benign and malignant thyroid disorders that need to be exactly attributed. The present study was performed to clarify the possible role of some trace elements (TEs) as cancer biomarker. For this aim thyroid tissue levels of copper (Cu), iron (Fe), iodine (I), rubidium (Rb), strontium (Sr), and zinc (Zn) were prospectively evaluated in malignant tumor and thyroid tissue adjacent to tumor of 41 patients with TMNs. Measurements were performed using energy-dispersive X-ray fluorescent analysis. Results of the study were additionally compared with previously obtained data for the same TEs in "normal" thyroid tissue. **F**rom results obtained, it was possible to conclude that the common characteristics of TMNs in comparison with "normal" thyroid and visually "intact" thyroid tissue adjacent to tumor were drastically reduced level of I. It was supposed that the drastically reduced level of I content in cancerous tissue could possibly be explored for differential diagnosis of benign and malignant thyroid nodules.

Introduction

Thyroid malignant nodules (TMNs) are the most common endocrine cancer and the fifth most frequently occurring type of malignancies [1-3]. The incidence of TMNs has increased worldwide over the past four decades. TMNs are divided into three main

histological types: differentiated (papillary and follicular thyroid cancer), undifferentiated (poorly differentiated and anaplastic thyroid cancer, and medullary thyroid cancer, arising from C cells of thyroid [3]. During the 20th century, there was the dominant opinion that TMNs is the simple consequence of iodine deficiency [4]. However, it was found that TMNs is a frequent disease even in those countries and regions where the population is never exposed to iodine shortage. Moreover, it was shown that iodine excess has severe consequences on human health and associated with the presence of TMNs [5-8]. It was also demonstrated that besides the iodine deficiency and excess many other dietary, environmental, and occupational factors are associated with the TMNs incidence [9-11]. Among these factors a disturbance of evolutionary stable input of many trace elements (TEs) in human body after industrial revolution plays a significant role in etiology of TMNs [12].

Besides iodine, many other TEs have also essential physiological functions [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of TEs depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the TEs may disturb the cell functions and may result in cellular proliferation, 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 TEs 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 TEs content with age in the thyroid of males and females were studied and age- and gender-dependence of some TEs was observed [25-41]. Furthermore, a significant difference between some TEs contents in colloid goiter, thyroiditis, and thyroid adenoma in comparison with normal thyroid was demonstrated [42-46].

To date, the etiology and pathogenesis of TMNs must be considered as multifactorial. The present study was performed to find out differences in TEs contents between the group of cancerous tissues, thyroid tissue adjacent to tumor, and "normal" thyroid (TEs as thyroid cancer biomarkers), as well as to clarify the role of some TEs in the etiology of TMNs. Having this in mind, the aim of this exploratory study was to examine differences in the content of copper (Cu), iron (Fe), iodine (I), rubidium (Rb), strontium (Sr), and zinc (Zn) in tumors and adjacent to tumor tissues of thyroids with TMNs, using a combination of non-destructive ^{109}Cd and ^{241}Am radionuclide-induced energy-dispersive X-ray fluorescent analysis, and to compare the levels of these TEs in two groups (tumor and adjacent to tumor tissues) of the cohort of TMNs samples. Moreover, for understanding a possible role of TEs in etiology and pathogenesis of TMNs, as well as thyroid cancer biomarkers, results of the study were compared with previously obtained data for the same TEs in "normal" thyroid tissue [42-46].

Material and Methods

All patients with TMNs (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 (MRRC), Obninsk.. 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. Tissue samples of tumor and visually intact tissue adjacent to tumor were taken from resected materials.

"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 studies were approved by the Ethical Committees of MRRC. 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. Informed consent was obtained from all individual participants included in the study

All tissue samples obtained from tumors and visually intact tissue adjacent to tumors were divided into two portions using a titanium scalpel to prevent contamination by TEs of stainless steel [47]. One was used for morphological study while the other was intended for TEs analysis. After the samples intended for TEs analysis were weighed, they were freeze-dried and homogenized [48].

To determine the contents of the TEs by comparison with known data for standard, aliquots of commercial, chemically pure compounds and synthetic reference materials were used [49]. Ten subsamples of the Certified Reference Material (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 dry homogenized thyroid tissue.

Details of the relevant facility for EDXRF determination of Cu, Fe, Rb, Sr, and Zn contents with ^{109}Cd radionuclide source, methods of analysis and the quality control of results were presented in our earlier publications concerning the ^{109}Cd-EDXRF analysis of human thyroid and prostate tissue [25,26,50,51].

Details of the relevant facility for EDXRF determination of I contents with ^{241}Am radionuclide source, methods of analysis and the quality control of results were presented in our earlier publication concerning the ^{241}Am-EDXRF analysis of human thyroid in norm and pathology [21].

All thyroid samples for TEs analysis were prepared in duplicate, and mean values of TEs contents were used in final calculation. Using Microsoft Office Excel software, a summary of the statistics, including, arithmetic mean, standard deviation of mean, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for TEs contents in nodular and adjacent tissue of thyroids with TMNs. Data for "normal" thyroid were taken from our previous publications [42-46]. The difference in the results between three groups of samples ("normal", "tumor", and "adjacent") was evaluated by the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

Results

Table 1 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 Cu, Fe, I, Rb, Sr, and Zn mass fraction in "normal", "tumor", and "adjacent" groups of thyroid tissue samples.

The ratios of means and the comparison of mean values of Cu, Fe, I, Rb, Sr, and Zn mass fractions in pairs of sample groups such as "normal" and "tumor", "normal" and "adjacent", and also "adjacent" and "tumor" are presented in Table 2, 3, and 4, respectively.

Discussion

As was shown before [21,25,26,50,51] good agreement of the Cu, Fe, I, Rb, Sr, and Zn contents in CRM IAEA H-4 samples analyzed by EDXRF with the certified data of this CRM indicates acceptable accuracy of the results obtained in the study of "normal", "tumor", and "adjacent" groups of thyroid tissue samples presented in Tables 1–4.

From Table 2, it is observed that in cancerous tissue the mass fraction of I and Zn are 23 times and 25%, respectively, lower whereas mass fractions of Cu and Rb are 3.4 and 1.4 times, respectively, higher than in normal

tissues of the thyroid. Thus, if we accept the TEs contents in thyroid glands in the "normal" group as a norm, we have to conclude that with a malignant transformation the Cu, I, Rb, and Zn in thyroid tissue significantly changed. In a general sense Cu, Fe, and Zn contents found in the "normal" and "adjacent" groups of thyroid tissue samples were very similar (Table 3). However, in the "adjacent" group mean mass fractions of I and Rb were 1.75 and 2.06 times, respectively, higher, whereas mean value of Sr content 4 times lower than in the "normal" group. Significant changes of tumor TEs contents in comparison with thyroid tissue adjacent to tumor were found only for I (decrease) and Sr (increase). In malignant tumor Sr contents were approximately 5.4 times higher, while I content 40 times lower than in "adjacent" group of tissue samples (Table 4). Thus, from obtained results it was possible to conclude that the common characteristics of TMNs in comparison with "normal" thyroid and visually "intact" thyroid tissue adjacent to malignant tumors were drastically reduced level of I (Tables 2 and 4).

Characteristically, elevated or reduced levels of TEs observed in thyroid nodules are discussed in terms of their potential role in the initiation and promotion of these thyroid lesions. In other words, using the low or high levels of the TEs in affected thyroid tissues researchers try to determine the role of the deficiency or excess of each TE in the etiology and pathogenesis of thyroid diseases. In our opinion, abnormal levels of many TEs in TMNs 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 TEs level in pathologically altered tissue is the reason for alterations or vice versa. According to our opinion, investigation of TEs contents in thyroid tissue adjacent to malignant nodules and comparison obtained results with TEs levels typical of "normal" thyroid gland may give additional useful information on the topic because these data show conditions of tissue in which TMNs were originated and developed.

Copper

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 [52-54]. Thus, Cu accumulation in thyroid parenchyma with age may be involved in oxidative stress, dwindling gland function, and increasing risk of goiter or cancer [25,26,31-34]. The significantly elevated level of Cu in thyroid malignant tumors and tissue adjacent to 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 TMNs.

Iodine

Nowadays it was well established that iodine deficiency or excess has severe consequences on human health and associated with the presence of TMNs [4-8][55-57]. In present study elevated level of I in thyroid tissue adjacent to malignant tumor and drastically reduced I mass fraction in cancerous tissue was found in comparison with "normal" thyroid.

Compared to other soft tissues, the human thyroid gland has higher levels of I, because this element plays an important role in its normal functions, through the production of thyroid hormones (thyroxin and triiodothyronine) which are essential for cellular oxidation, growth, reproduction, and the activity of the central and autonomic nervous system. As was shown in

Table 1. Some statistical parameters of Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid and thyroid cancer ((tumor and "intact" thyroid tissue adjacent to tumor)

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal	Cu	4.23	1.52	0.18	0.50	7.50	4.15	1.57	7.27
thyroid	Fe	222	102	11	47.1	512	204	65.7	458
	I	1618	1041	108	110	5150	1505	220	3939
	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	Cu	14.5	9.4	2.6	4.00	32.6	10.9	4.21	31.4
(tumor)	Fe	238	184	30	54	893	176	55.0	680
	I	71.6	72.5	11.6	2.00	341	64.0	2.19	237
	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
Cancer	Cu	8.08	3.15	1.58	4.90	12.1	7.65	5.01	11.9
(adjacent	Fe	239	137	26	95.2	753	201	104	584
tissue)	I	2839	1335	240	587	6571	2652	827	5675
	Rb	18.6	16.7	3.2	5.00	67.0	12.0	5.72	65.6
	Sr	1.16	0.29	0.14	0.83	1.40	1.20	0.84	1.40
	Zn	109	55	11	20.4	272	109	29.1	213

M – arithmetic mean, SD – standard deviation, SEM – standard error of mean, Min – minimum value, Max – maximum value, P 0.025 – percentile with 0.025 level, P 0.975 – percentile with 0.975 level.

Table 2. Differences between mean values (M±SEM) of Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid and thyroid cancer ((tumor)

Element	Thyroid tissue				Ratio
	Normal thyroid	Cancer (tumor)	Student's t-test *p*£	U-test *p*	Tumor/Normal
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
I	1618±108	71.6±11.6	**0.00000000001**	**≤0.01**	0.044
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.

Table 3. Differences between mean values (M±SEM) of Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid and "intact" thyroid tissue adjacent to tumor

Element	Thyroid tissue				Ratio
	Normal thyroid	Cancer (adjacent)	Student's t-test *p*£	U-test *p*	Adjacent/Normal
Cu	4.23±0.18	8.08±1.58	0.092	**≤0.05**	1.91
Fe	222±11	239±26	0.542	>0.05	1.08
I	1618±108	2839±240	**0.000033**	**≤0.01**	1.75
Rb	9.03±0.66	18.6±3.2	**0.0068**	**≤0.01**	2.06
Sr	4.55±0.37	1.16±0.14	**0.00000000001**	**≤0.01**	0.25
Zn	112±5	109±11	0.778	>0.05	0.97

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

Table 4. Differences between mean values (M±SEM) of, Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in thyroid cancer and "intact" thyroid tissue adjacent to tumor

Element	Thyroid tissue				Ratio
	Cancer (adjacent)	Cancer (tumor)	Student's t-test *p*£	U-test *p*	Adjacent/Tumor
Cu	8.08±1.58	14.5±2.6	0.051	**≤0.05**	1.79
Fe	239±26	238±30	0.978	>0.05	1.00
I	2839±240	71.6±11.6	**0.00000000001**	**≤0.01**	0.025
Rb	18.6±3.2	12.4±0.79	0.072	>0.05	0.67
Sr	1.16±0.14	6.25±1.63	**0.0051**	**≤0.01**	5.39
Zn	109±11	84.3±9.2	0.083	>0.05	0.77

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

present study, malignant transformation is accompanied by a significant loss of tissue-specific functional features, which leads to a drastically reduction in I content associated with functional characteristics of the human thyroid tissue. Because the malignant part of gland stopped to produce thyroid hormones, the rest "intact" part of thyroid tries to compensate thyroid hormones deficiency and work more intensive than usual. The intensive work may explain elevated level of I in thyroid tissue adjacent to malignant tumor.

Drastically reduced level of I content in cancerous tissue could possibly be explored for differential diagnosis of benign and malignant thyroid nodules, because, as was found in our ealier studies, thyroid benign trasformation (goiter, thyroiditis, and adenoma) is accompanied by a little loss of I accumulation [42-46].

Rubidium

There is very little information about Rb effects on thyroid function. Rb as a monovalent cation Rb+ is transfered through membrane by the Na+K+-ATPase pump like K+ and concentrated in the intracellular space of cells. Thus, Rb seems to be more intensivly concentrated in the intracellular space of cells. The sourse of Rb elevated level in tumor and adjacent to tumor tissue may be Rb environment overload. The excessive Rb intake may result a replacement of medium potassium by Rb, which effects on iodide transport and iodoaminoacid synthesis by thyroid [58]. The sourse of Rb increase in TMNs tissue may be not only the excessive intake of this TE in organism from the environment, but also changed Na+K+ -ATPase or H+K+ - ATPase pump membrane transport systems for monovalent cations, which can be stimulated by endocrin system, including thyroid hormones [59]. It was found also that Rb has some function in immune responce [60] and that elevated concentration of Rb could modulate proliferative responses of the cell, as was shown for bone marrow leukocytes [61]. These data partially clarify the possible role of Rb in etiology and pathogenesis of TMNs.

Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only six TEs (Cu, Fe, I, Rb, Sr, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of TEs investigated in "normal" thyroid and in pathologically altered tissue. Secondly, the sample size of TMNs group was relatively small and prevented investigations of TEs contents in this group using differentials like gender, histological types of TMNs, tumor functional activity, stage of disease, and dietary habits of patients with TMNs. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on many TEs level alteration in malignant tumor and adjacent to tumor tissue and shows the necessity to continue TEs research of TMNs.

Conclusion

In this work, TEs analysis was carried out in the tissue samples of TMNs using EDXRF. It was shown that EDXRF with using ^{109}Cd and ^{241}Am radionuclide sources is an adequate analytical tool for the non-destructive determination of Cu, Fe, I, Rb, Sr, and Zn content in the tissue samples of human thyroid in norm and pathology, including needle-biopsy specimens. It was observed that in cancerous tissue the mass fraction of I and Zn were 23 times and 25%, respectively, lower whereas mass fractions of Cu and Rb were 3.4 and 1.4 times, respectively, higher than in normal tissues of the thyroid. In a general sense Cu, Fe, and Zn contents found in the "normal" and "adjacent" groups of thyroid tissue samples were very similar. However, in the "adjacent" group mean mass fractions of I and Rb were 1.75 and 2.06 times, respectively, higher, while mean value of Sr content was 4 times lower than in the "normal" group. In malignant tumor Sr contents were approximately 5.4 times higher, while I content 40 times lower than in "adjacent" group of tissue samples. Thus, from results obtained, it was possible to conclude that the common characteristics of TMNs in comparison with "normal" thyroid and visually

"intact" thyroid tissue adjacent to nodules were drastically reduced level of I. It was supposed that the drastically reduced level of I content in cancerous tissue could possibly be explored for differential diagnosis of benign and malignant thyroid nodules.

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Conflict of Interest

The author has not declared any conflict of interests.

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