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Possible Role of Inadequate Quantities of Intra-Thyroidal Bromine, Rubidium and Zinc in the Etiology of Female Subclinical Hypothyroidism

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Abstract

Background: Subclinical hypothyroidism does affect fertility. The prevalence of subclinical hypothyroidism is 10-15 times more common in women than in men. Trace elements play important roles in thyroid function.

Objective: The aim of this exploratory study was to evaluate whether significant differences of trace element contents exists between female and male thyroids and how they can be related to the etiology of subclinical hypothyroidism.

Methods: Thyroid tissue levels of bromine (Br), coper (Cu), iron (Fe), rubidium (Rb), strontium Sr) and zinc (Zn) were prospectively evaluated in 105 healthy persons (33 females and 72 males). Measurements were performed using 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 the Br thyroid content of females was significantly higher than that of males, while the Rb and Zn contents were lower.

Conclusion: Involvement of inadequate contents of intra-thyroidal Br, Rb and Zn in the etiology of female subclinical hypothyroidism may be assumed.

Keywords: *Subclinical Hypothyroidism; Female Thyroid; Trace Elements; Energy-Dispersive X-Ray Fluorescent Analysis*

Abbreviations

SCH: Subclinical Hypothyroidism; Tel: Trace Elements; EDXRF: Energy Dispersive X-Ray Fluorescent Analysis; SRM: Standard Reference Material; CRM: Certified Reference Material; BSS: Biological Synthetic Standards

Introduction

Adequate thyroid function is important to maintain normal reproduction, because thyroid dysfunction affects fertility in various ways resulting in abnormal ovulatory cycles, luteal phase defects, high prolactin levels, and sex hormone imbalances [1,2]. Therefore, normal thyroid function is necessary for fertility, and to sustain a healthy pregnancy [2]. From large population studies, which measured thyroid function, and systematic reviews of this subject carried out in the 1990s to 2010s, it is known that untreated hypothyroidism is a common condition all over the world [2-11]. The prevalence of subclinical hypothyroidism (SCH) is between 1% and 10% in different countries [2-11] and almost everywhere it is 10 - 15 times more common in women than in men [4,10]. Form such a great gender-related difference in the prevalence of SCH arises a question about a specific sensitivity of female thyroid tissue to some external and internal factors.

Although the etiology of SCH and other thyroidal disorders is unknown in detail, several risk factors including deficiency or excess of such micronutrients as iodine (I) has been well identified [12-23]. Besides I involved in thyroid function, other trace elements (TE) also play important roles such as stabilizers, structural elements, maintenance and regulation of cell function, gene regulation, enzyme cofactors, activation or inhibition of enzymatic reactions, normal peripheral utilization of thyroid hormones and regulation of cell membrane function [24]. Essential or toxic properties of TE depend on tissue-specific need or tolerance, respectively [25]. Both TE deficiencies as well as overexposures may disturb the thyroidal cell functions [25]. Moreover, TE affect each other's homeostasis as some of them are sharing and competing for protein transporters and binding proteins such as metallothioneins. The effect of these interactions can be synergistic or antagonistic. Therefore, besides measuring only total amounts of TE, the interrelationship of TE should be taken into account to allow for a more comprehensive description of the TE impact on thyroid health status.

The reliable data on TE mass fractions in normal human thyroid separately for female and male gland is apparently extremely limited. There are a few studies regarding TE content in human thyroid, using chemical techniques and instrumental methods [26-31]. However, the majority of these data are based on measurements of processed tissue and in many studies tissue samples are ashed before analysis. In other cases, thyroid samples are treated with solvents (distilled water, ethanol etc.) and then are dried at a high temperature for many hours. There is evidence that certain quantities of TE are lost as a result of such treatment [32-34]. Moreover, only a few of these studies employed quality control using certified/standard reference materials (CRM/SRM) for determination of the TE mass fractions. Sample-nondestructive technique such as energy dispersive X-ray fluorescent (EDXRF) is good alternatives for multi-element determination in the samples of thyroid parenchyma.

This work had three aims. The primary purpose of this study was to determine reliable values for such TE as bromine (Br), coper (Cu), iron (Fe), rubidium (Rb), strontium (Sr), and zinc (Zn) contents in intact (normal) thyroid gland of apparently healthy persons using EDXRF analysis. The second aim was to compare the levels of TE in the thyroid tissue of females and males. The final aim was to find and compare the inter-correlations of TE in normal thyroid of females and males.

All studies were approved by the Forensic Medicine Department of Obninsk City Hospital and the Ethical Committee of the Medical Radiological Research Center.

Method

Samples of the human thyroid were obtained from randomly selected autopsy specimens of 33 females (European-Caucasian, aged 3.5 to 87 years) and 72 males (European-Caucasian, aged 2.0 to 80 years). All the deceased were citizens of Obninsk and had undergone routine autopsy at the Forensic Medicine Department of City Hospital, Obninsk. Age ranges for subjects were divided into two age groups, with group 1 (≤ 40 years), and group 2 (> 40 years). For females in group 1 (n = 11) mean age (± standard error of mean, SEM) was 30.9 ± 3.1 years and in group 2 (n = 22) mean age was 66.3 ± 2.7 years. For males in group 1 (n = 36) mean age was 22.5 ± 1.4 years and in group 2 (n = 36) mean age was 52.4 ± 2.4 years. These groups were selected to reflect the condition of thyroid tissue in the children, teenagers, young adults and first period of adult life (group 1) and in the second period of adult life as well as in old age (group 2). The available clinical data were reviewed for each subject. None of the subjects had a history of an intersex condition, endocrine disorder, or other chronic disease that could affect the normal development of the thyroid. None of the subjects were receiving medications or used any supplements known to affect thyroid trace element contents. The typical causes of sudden death of most of these subjects included trauma or suicide and also acute untreated illness (cardiac insufficiency, stroke, embolism of pulmonary artery, alcohol poisoning).

All right lobes of thyroid glands were divided into two portions using a titanium scalpel [35]. One tissue portion was reviewed by an anatomical pathologist while the other was used for the TE content determination. A histological examination was used to control the age norm conformity as well as the unavailability of microadenomatosis and latent cancer. After the samples intended for TE analysis were weighed, they were freeze-dried and homogenized [36-38]. The pounded sample weighing about 8 mg was applied to the piece of Scotch tape serving as an adhesive fixing backing.

To determine the contents of the TE by comparison with known data for standard, aliquots of commercial, chemically pure compounds and synthetic reference materials were used [39]. 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 prostate tissue [40-43].

Statistical Analysis

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 thyroid tissue samples of females and males. The difference in the results between females and males (age group 1 and 2 combined), as well as between females and males separately in age group 1 and group 2 was evaluated by the parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test.

Results

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

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

***Table 1:** Energy Dispersive X-Ray Fluorescence data for 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)*
Mean: Arithmetical Mean; SD: Standard Deviation; C: Certified Values; N: Non-Certified Values.

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, and Zn mass fraction in normal thyroid tissue of female and male.

Gender	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Males	Br	10.8	10.0	1.3	1.90	54.4	8.05	2.33	42.0
n = 72	Cu	4.25	1.48	0.20	1.10	7.50	4.15	1.78	7.39
	Fe	221	102	13	47.1	502	224	58.4	419
	Rb	10.05	6.96	0.89	1.80	42.9	8.60	2.65	27.5
	Sr	4.52	3.27	0.43	0.10	13.7	3.55	0.44	12.4
	Zn	122	40.9	5.2	35.4	221	115	57.2	201
Females	Br	20.4	13.4	2.6	1.40	54.1	16.3	4.52	52.2
n = 33	Cu	4.18	1.72	0.43	0.50	6.50	4.05	1.18	6.50
	Fe	223	104	21	84.0	512	191	87.6	442
	Rb	6.64	2.47	0.48	2.20	12.8	6.38	3.08	11.7
	Sr	4.67	3.11	0.78	0.65	10.9	4.40	0.82	10.8
	Zn	89.0	43.0	8.4	6.10	166	88.1	6.16	156

***Table 2:** Some statistical parameters of Br, Cu, Fe, Rb, Sr, and Zn mass fractions (mg/kg, dry mass basis) in normal thyroid tissue of females and males.*
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.

The comparison of our results with published data for Br, Cu, Fe, Rb, Sr, and Zn mass fraction in normal human thyroid is shown in table 3.

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)**	Females and males (combined) M ± SD
Br	18.1 (11)	5.12 (44) [26]	284 ± 44 (14) [27]	13.9 ± 12.0
Cu	6.1 (57)	1.42 (120) [28]	220 ± 22 (10) [29]	4.2 ± 1.5
Fe	252 (21)	56 (120) [28]	2444 ± 700 (14) [27]	222 ± 102
Rb	12.3 (9)	≤ 0.85 (29) [30]	294 ± 191 (14) [27]	9.0 ± 6.2
Sr	0.73 (9)	0.55 ± 0.26 (21) [31]	46.8 ± 4.8 (4) [29]	4.6 ± 3.2
Zn	118 (51)	32 (120) [28]	820 ± 204 (14) [27]	112 ± 44

***Table 3:** Median, minimum and maximum value of means of Br, Cu, Fe, Rb, Sr, and Zn contents in normal human thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis)*
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, and Zn mass fractions in normal thyroid of females and males are presented in table 4. Because, in our previous studies age-dependents of many chemical elements in thyroid gland was found [44-46], the comparison between TE contents in thyroid of females and males separately in age group 1 and in age group 2 was also performed (Tables 5 and 6).

Element	Thyroid tissue				Ratio
	Males 2.0 - 80 years n = 72	Females 3.5 - 87 years n = 33	Student's t-test $p \leq$	U-test p	Females to Males
Br	10.8 ± 1.3	20.4 ± 2.6	**0.00197**	≤ **0.01**	1.89
Cu	4.25 ± 0.20	4.18 ± 0.43	0.873	>0.05	0.98
Fe	221 ± 13	223 ± 21	0.946	>0.05	1.01
Rb	10.05 ± 0.89	6.64 ± 0.48	**0.00116**	≤ **0.01**	0.66
Sr	4.52 ± 0.43	4.67 ± 0.78	0.867	>0.05	1.03
Zn	122 ± 5	89.0 ± 8.4	**0.00167**	≤ **0.01**	0.73

Table 4: *Differences between mean values (M ± SEM) of Br, Cu, Fe, Rb, Sr, and Zn mass fractions (mg/kg, dry mass basis) in normal thyroid tissue of males and females.*
*M: Arithmetic Mean; SEM: Standard Error of Mean; Statistically significant values are in **bold.***

Element	Thyroid tissue				Ratio
	Males (MG1) n = 44	Females (FG1) n = 11	Student's t-test $p \leq$	U-test p	FG1/MG1
Br	9.20 ± 1.25	13.1 ± 2.5	0.184	>0.05	1.42
Cu	4.03 ± 0.24	4.01 ± 0.60	0.973	>0.05	1.00
Fe	231 ± 16	172 ± 26	0.069	≤**0.05**	0.74
Rb	10.1 ± 1.2	5.30 ± 0.55	**0.00067**	≤**0.01**	0.52
Sr	4.53 ± 0.49	5.29 ± 1.12	0.548	>0.05	1.17
Zn	116 ± 7	65.6 ± 12.7	**0.0029**	≤**0.01**	0.57

Table 5: *Differences between mean values (M ± SEM) of Br, Cu, Fe, Rb, Sr, and Zn mass fractions (mg/kg, dry mass basis) in normal thyroid tissue of males and females aged ≤ 40 years.*
*M: Arithmetic Mean; SEM: Standard Error of Mean; Statistically Significant Values are in **bold.***

Element	Thyroid tissue				Ratio
	Males (MG2) n = 28	Females (FG2) n = 22	Student's t-test $p \leq$	U-test p	FG2/MG2
Br	13.1 ± 2.6	24.7 ± 3.5	**0.011**	≤ **0.01**	1.89
Cu	4.59 ± 0.33	4.45 ± 0.61	0.844	> 0.05	0.97
Fe	204 ± 21	263 ± 27	0.098	> 0.05	1.29
Rb	10.0 ± 1.3	7.62 ± 0.64	0.106	≤ **0.05**	0.76
Sr	4.49 ± 0.83	3.63 ± 0.86	0.483	> 0.05	0.81
Zn	134 ± 8	106 ± 9	**0.030**	≤ **0.01**	0.79

Table 6: *Differences between mean values (M ± SEM) of Br, Cu, Fe, Rb, Sr, and Zn mass fractions (mg/kg, dry mass basis) in normal thyroid tissue of males and females aged > 40 years.*
*M: Arithmetic Mean; SEM: Standard Error of Mean; Statistically Significant Values are in **bold.***

The data of inter-correlation calculations (values of r – coefficient of correlation) including all TE identified by us in thyroid of females and males separately are presented in table 7.

Gender	Element	Br	Cu	Fe	Rb	Sr	Zn
Male	Br	**1.00**	0.285[a]	0.150	0.299[a]	0.302[a]	0.408[b]
	Cu	0.285[a]	**1.00**	0.413[b]	0.273[a]	-0.147	0.218
	Fe	0.150	0.413[b]	**1.00**	0.197	0.072	0.133
	Rb	0.299[a]	0.273[a]	0.197	**1.00**	0.112	0.098
	Sr	0.302[a]	-0.147	0.072	0.112	**1.00**	0.174
	Zn	0.408[b]	0.218	0.133	0.098	0.174	**1.00**
Female	Br	**1.00**	0.173	0.255	0.062	-0.200	0.023
	Cu	0.173	**1.00**	0.048	0.009	0.249	0.024
	Fe	0.255	0.048	**1.00**	0.185	-0.349[a]	0.213
	Rb	0.062	0.009	0.185	**1.00**	-0.116	0.481[b]
	Sr	-0.200	0.249	-0.349[a]	-0.116	**1.00**	-0.001
	Zn	0.023	0.024	0.213	0.481[b]	-0.001	**1.00**

Table 7: *Intercorrelations of the trace element mass fractions in the normal thyroid of males and females (r – coefficient of correlation).*
Statistically significant values: [a] $p \leq 0.05$, [b] $p \leq 0.01$.

Discussion

Precision and accuracy of results: Good agreement of the Br, Cu, Fe, Rb, Sr, and Zn contents analyzed by EDXRF with the certified data of CRM IAEA H-4 (Table 1) indicates an acceptable accuracy of the results obtained in the study of TE of the thyroid samples presented in tables 2-7.

The mean values and all selected statistical parameters were calculated for six TE (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 females and males thyroid 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 [26-31]. Data cited in Table 3 also includes samples obtained from patients who died from different non-endocrine diseases. A number of values for TE mass fractions were not expressed on a dry mass basis by the authors of the cited references. However, we calculated these values using published data for water (75%) [47] and ash (4.16% on dry mass basis) [48] contents in thyroid of adults.

The range of means of Br, Cu, Fe, Rb, Sr, and Zn level reported in the literature for normal human thyroid vary widely (Table 3). This can be explained by a dependence of TE content on many factors, including the region of the thyroid, from which the sample was taken, age, gender, ethnicity, and mass of the gland. 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 insufficient quality control of results in these studies.

Gender-related differences: Strongly pronounced differences in Br, Rb, and Zn mass fractions were observed between female and male thyroid (Table 4). The mean Br mass fraction in female thyroids was almost 2 times higher while the means of Rb and Zn mass fractions were respectively 34% and 27% lower than in male thyroids. During the first 40 years of life (Age group 1) the situation with TE contents in female thyroids was some different than that for older females. In Age group 1 no statistically significant difference between the Br content of female and male thyroids was found, but differences between their Rb and Zn contents were detected (Table 5). In Age group 1 of females with mean age 30.9 years the Rb and Zn thyroid contents were almost half those in thyroids of males from the same age group. Moreover, in this age group a modest but statistically significant reduced level of the Fe mass fraction in female thyroids was observed using the non-parametric Wilcoxon-Mann-Whitney U-test. For ages over 40 years (Age group 2) a statistically significant difference between the Br content in female and male thyroid was observed and differences for the Rb and Zn contents remained as for Age group 1 (Table 6). In Age group 2 a difference between the Fe content in thyroids of females and males, previously found in the Age group 1, was no longer evident.

A significant direct inter-correlation between Br and Cu, Br and Rb, Br and Sr, Br and Zn, Cu and Fe, and Cu and Rb mass fractions was seen in male thyroids (Table 7). In female thyroids all those correlations between TE found in male thyroids were not observed but two other different correlations were found: a direct correlation, Zn and Rb, and an inverse correlation, Fe and Sr.

Because the prevalence of SCH is 10 - 15 times greater in women than in men [4,10], we can accept that the levels and relationships of TE mass fractions in male thyroids as more suitable (perhaps optimal) for normal function of the gland. If so, we have to conclude that up to age 40 years there is a significant deficiency of Rb and Zn contents in female thyroid parenchyma, accompanied by a modest deficiency of Fe. In age over 40 significant deficiencies of Rb and Zn contents in female thyroids persist, while the Fe deficiency disappears and an excess of Br is now seen. Moreover, because inter-correlations of TE contents reflect their relationships one can deduce that relationships of TE in female thyroids are certainly less optimal that in males.

Bromine: Br is one of the most abundant and ubiquitous of TE in the biosphere. Inorganic bromide compounds, especially potassium bromide (KBr), sodium bromide (NaBr), and ammonium bromide (NH4Br), are frequently used as sedatives in Russia [49]. This may be the reason for elevated levels of Br in female thyroids, because females particularly if aged over 40 years use sedatives more intensively than males. Inorganic bromide exerts therapeutic as well as toxic effects. An enhanced intake of bromide could interfere with the metabolism of iodine at the whole-body level, for both elements have similar chemical properties, and are adjacent halogens. So in the thyroid gland the biological behavior of bromide is similar to that of iodide [50]. Therefore, an excessive Br level in the thyroid of elderly females might inhibit thyroid hormonal synthesis.

Iron: The low Fe level in the thyroid of young women compared with men can be attributed to physiological characteristics of the female body related to reproduction and pre-menopausal physiology [51].

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 [52]. Rb has some function in immune responce [53], probably by supporting cell differentiation [54]. Both 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. Thus, the low Rb level in the thyroid of women compared with men might reflect the reduced ratio "Volume of thyroid cells/Volume of follicular colloid" in the female thyroid. Thyroid function depends in part on the total volume of active thyroid cells. From this it might be concluded that the reduced level of active cells in the thyroids of women compared to men increases risk of hypothyroidism.

Zinc: Zn is a most essential TE for humans. Today more than 300 proteins and over 100 DNA-binding proteins that require Zn have been classified. Zn is required for the synthesis of thyroid hormones, and deficiency of this TE can result in hypothyroidism [55,56]. Thus, a Zn deficiency in female thyroid parenchyma observed in the present study may be one of the reasons for the higher incidence of SCH in females in comparison with males.

Each of the TE is distinct in its primary mode of action. Moreover, there are several forms of synergistic action of the TE as a part of intracellular metabolism. Thus, in addition to TE content gender-related differences of relationships between them (inter-correlations) might be also involved in the etiology of SCH.

Conclusion

Our data indicate that there is a statistically significant gender-related difference between TE levels in the thyroid tissue of females and males. The Br mass fraction is higher while the Rb and Zn mass fractions are lower in female thyroids compared with those in male thyroids. Subclinical hypothyroidism is a multietiological and multifactorial complex condition. The complete understanding of the role of inadequate levels of some TE in thyroid parenchyma in the etiology of SCH requires a global vision of their different mechanisms of action, which is not yet possible with the present state of knowledge. However, from the results of our study it follows that an involvement of inadequate contents of intra-thyroidal Br, Rb and Zn in the etiology of female SCH may be assumed.

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Conflict of Interest

There is no any financial interest or any conflict of interest

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