ISSN: 2755-0168

Journal of Biotechnology & Bioinformatics Research

Scientific Research and Community

Research Article

Open Access

Content of Fifty Trace Elements in Thyroid Benign Nodules and Thyroid Tissue adjacent to Nodules investigated using Neutron Activation Analysis and Inductively Coupled Plasma Mass Spectrometry

Vladimir Zaichick

Radionuclide Diagnostics Department, Medical Radiological Research Centre, Russia

ABSTRACT

Thyroid benign nodules (TBNs) are the most common diseases of this endocrine gland and are common worldwide. The etiology and pathogenesis of TBNs must be considered as multifactorial. The present study was performed to clarify the role of some trace elements (TEs) in the etiology of these thyroid disorders. Thyroid tissue levels of fifty TEs were prospectively evaluated in nodular tissue and tissue adjacent to nodules of 79 patients with TBNs. Measurements were performed using a combination of non-destructive instrumental neutron activation analysis and destructive method such as inductively coupled plasma mass spectrometry. Results of the study were additionally compared with previously obtained data for the same TEs in "normal" thyroid tissue. This study provides evidence on many TEs level alteration in nodular and adjacent to nodule tissue and shows the necessity to continue TEs research of TBNs.

***Corresponding author**

Prof. Dr. Vladimir Zaichick, PhD, DSc, CChem, FRSC, Medical Radiological Research Centre, Koroleyv St. 4, Obninsk 249036, Phone: +7(48439)60289, Russia. E-mail: vzaichick@gmail.com

Received: January 01, 2022; **Accepted:** January 07, 2022; **Published:** January 11, 2022

Keywords: Trace Elements, Thyroid, Thyroid Benign Nodules, Neutron Activation Analysis, Inductively Coupled Plasma Mass Spectrometry

Introduction

Thyroid benign nodules (TBNs) are universally encountered and frequently detected by palpation during a physical examination, or incidentally, during clinical imaging procedures. TBNs include non-neoplastic lesions, for example, colloid goiter and thyroiditis, as well as neoplastic lesions such as thyroid adenomas [1-3]. For over 20th century, there was the dominant opinion that TBNs is the simple consequence of iodine deficiency. However, it was found that TBNs is a frequent disease even in those countries and regions where the population is never exposed to iodine shortage [4]. Moreover, it was shown that iodine excess has severe consequences on human health and associated with the presence of TBNs [5-8]. It was also demonstrated that besides the iodine deficiency and excess many other dietary, environmental, and occupational factors are associated with the TBNs 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 TBNs [12]. Besides iodine, many other TEs have also essential physiological functions. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of TEs depend on tissue-specific need or tolerance, respectively. 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 [42-49].

To date, the etiology and pathogenesis of TBNs must be considered as multifactorial. The present study was performed to find out differences in TEs contents between the group of nodular tissues and tissue adjacent to nodules, as well as to clarify the role of some TEs in the etiology of TBNs. Having this in mind, the aim of this exploratory study was to examine differences in the content of silver (Ag), aluminum (Al), arsenic (As), gold (Au), boron (B),, beryllium (Be), bismuth (Bi), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), cesium (Cs), dysprosium (Dy), iron (Fe), erbium (Er), europium (Eu), gallium (Ga), gadolinium (Gd), mercury (Hg), holmium (Ho), iridium (Ir), lanthanum (La), lithium (Li), lutecium (Lu), manganese (Mn), molybdenum (Mo), niobium (Nb), neodymium (Nd), nickel (Ni), lead (Pb), palladium (Pd), praseodymium (Pr), platinum (Pt), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), samarium (Sm), tin

(Sn), terbium (Tb), tellurium (Te), thorium (Th), titanium (Ti), thallium (Tl), thulium (Tm), uranium (U), yttrium (Y), ytterbium (Yb), zinc (Zn), and zirconium (Zr) in nodular and adjacent to nodules tissues of thyroids with TBNs, using a combination of non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) and destructive method such as inductively coupled plasma mass spectrometry (ICP-MS), and to compare the levels of these TEs in two groups (nodular and adjacent to nodules tissues) of the cohort of TBNs samples. Moreover, for understanding a possible role of TEs in etiology and pathogenesis of TBNs results of the study were compared with previously obtained data for the same TEs in "normal" thyroid tissue [42-49].

Material and Methods

All 79 patients suffered from TBNs (46 patients with colloid goiter, mean age M±SD was 48±12 years, range 30-64; 19 patients with thyroid adenoma, mean age M±SD was 41±11 years, range 22-55; and 14 patients with thyroiditis, mean age M±SD was 39±9 years, range 34-50) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre (MRRC), Obninsk. The group of patients with thyroiditis included 8 persons with Hashimoto's thyroiditis and 6 persons with Riedel's Struma. 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 TEs contents. For all patients the diagnosis has been confirmed by clinical and morphological/histological results obtained during studies of biopsy and 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. Titanium tools were used for biopsy, getting tissue samples from resected materials, and sample preparation to prevent contamination by many alloy metals from stainless steel [50]. All tissue samples obtained from nodular tissue and visually "normal" tissue adjacent to nodules were divided into two portions. 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 [51].

The pounded samples weighing about 10 mg (for biopsy) and 100 mg (for resected materials) were used for TEs measurement by INAA-LLR. The content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn were determined by INAA-LLR using a vertical channel of the Water-Water-Research nuclear reactor (Branch of Karpov Institute, Obninsk). After non-destructive INAA-LLR investigation the thyroid samples were used for ICP-MS. The samples were decomposed in autoclaves and aliquots of solutions were used to determine the Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr mass fractions by ICP-MS using an ICP-MS Thermo-Fisher "X-7" Spectrometer (Thermo Electron, USA). Information detailing with the NAA-LLR and ICP-MS methods used and other details of the analysis were presented in our earlier publications concerning TE contents in human thyroid [29,30,35], prostate [52-57], and scalp hair [58].

To determine contents of the TEs by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [59]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten sub-samples of certified reference material (CRM) IAEA H-4 (animal muscle) and five sub-samples of CRM of the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results

A dedicated computer program for INAA-LLR mode optimization was used [60]. All thyroid samples were prepared in duplicate, and mean values of TEs contents were used in final calculation. Mean values of TEs contents were used in final calculation for the Ag, Co, Cr, Hg, Rb, Sb, Se, and Zn mass fractions measured by INAA-LLR and ICP-MS methods. 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 TBNs. Data for TEs content in "normal" thyroid were taken from our previous publications [42-49]. The difference in the results between three groups of samples ("normal", "nodular", 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 Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Fe, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Sc, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr mass fraction in nodular and adjacent to nodules tissue of thyroid with TBN ("nodular" and "adjacent" group of thyroid tissue samples).

The ratios of means and the comparison of mean values of Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Er, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, and Zn mass fractions in pairs of sample groups such as "normal" and "nodular", "normal" and "adjacent", and also "adjacent" and "nodular" are presented in Table 2, 3, and 4, respectively.

Table 1: Some statistical parameters of 50 trace element mass fraction (mg/kg, dry mass basis) in) in nodular and adjacent tissue of thyroid benign nodules (TBN)

Element	Nodular tissue			Adjacent tissue		
	M	SD	Range	M	SD	Range
Ag	0.192	0.199	0.00200-0.842	0.473	0.663	0.0470-3.31
Al	27.3	23.6	6.60-95.1	25.7	14.2	16.1-46.7
As	<0.004	-	-	<0.004	-	-
Au	0.0166	0.0194	0.00300-0.0709	≤0.0055	-	<0.0050-0.0060
B	4.65	15.0	0.810-85.2	1.70	0.61	1.00-2.10
Be	0.00090	0.00113	0.000200-0.00600	0.00053	0.00004	0.00050-0.00055
Bi	0.0706	0.0845	0.00390-0.422	0.478	0.453	0.0878-1.13
Cd	1.55	1.68	0.126-6.39	2.46	1.49	0.608-4.17
Ce	0.0181	0.0176	0.00310-0.0696	0.488	0.938	0.0080-1.90
Co	0.0576	0.0324	0.0150-0.159	0.0733	0.0979	0.00510-0.594
Cr	1.17	1.19	0.0750-7.30	0.614	0.650	0.0180-3.14
Cs	0.0320	0.0471	0.00760-0.205	0.0132	0.0058	0.00820-0.0195
Dy	<0.005	-	-	<0.005	-	-
Er	0.00303	0.00328	0.00100-0.0138	<0.001	-	-
Eu	<0.001	-	-	<0.001	-	-
Fe	430	566	52.3-2734	217	141	41.5-620
Ga	0.0211	0.0081	0.0100-0.0340	≤0.024	-	<0.020-0.027
Gd	<0.001	-	-	≤0.0077	-	<0.0030-0.0152
Hg	1.15	1.04	0.100-5.20	1.39	0.94	0.0140-4.68
Ho	<0.0002	-	-	<0.0002	-	-
Ir	<0.0003	-	-	≤0.0013	-	<0.0003-0.0030
La	0.00939	0.00882	0.00170-0.0356	≤0.088	-	<0.0030-0.321
Li	0.0295	0.0151	0.00730-0.0680	0.0350	0.0219	0.0175-0.0666
Lu	<0.0002	-	-	<0.0002	-	-
Mn	1.81	1.41	0.100-6.12	1.78	1.65	0.100-5.83
Mo	0.193	0.121	0.0460-0.627	0.127	0.061	0.0542-0.199
Nb	<0.013	-	-	<0.013	-	-
Nd	0.0134	0.0075	0.00310-0.0331	≤0.048	-	<0.0020-0.174
Ni	2.89	2.52	0.130-10.4	1.71	1.43	0.520-3.30
Pb	1.31	2.27	0.120-9.30	0.83	0.71	0.240-1.70
Pd	<0.012	-	-	<0.025	-	-
Pr	0.00389	0.00335	0.000530-0.0131	≤0.017	-	<0.0010-0.0637
Pt	<0.0002	-	-	<0.0002	-	-
Rb	9.50	4.23	2.50-22.1	10.4	4.3	4.90-20.0
Sb	0.121	0.108	0.00238-0.466	0.131	0.174	0.00760-0.757
Sc	0.0239	0.0383	0.000200-0.150	0.0058	0.0147	0.00020-0.0654
Se	3.20	2.92	0.720-13.8	1.93	0.86	0.647-4.34
Sm	0.00171	0.00181	0.000400-0.00800	≤0.0011	-	<0.0010-0.00120
Sn	0.0516	0.0399	0.0143-0.172	0.135	0.119	0.0438-0.309
Tb	<0.0001	-	-	≤0.00065	-	<0.00020-0.00110
Te	<0.007	-	-	<0.007	-	-
Th	0.0104	0.0155	0.00200-0.0783	<0.002	-	-
Ti	<0.4	-	-	<0.4	-	-
Tl	0.00190	0.00109	0.000520-0.00540	≤0.0036	-	<0.0012-0.0095
Tm	<0.0003	-	-	<0.0003	-	-
U	0.00116	0.00059	0.000380-0.00240	0.00180	0.00098	0.00100-0.00300

Y	0.0110	0.0108	0.00310-0.0361	0.0088	0.0063	0.0030-0.0160
Yb	0.000275	0.000133	0.00020-0.00070	<0.0002	-	-
Zn	117.7	48.7	47.0-264	105	68	34.2-344
Zr	0.0733	0.0444	0.0310-0.205	≤0.29	-	<0.06-0.696

M – arithmetic mean, SD – standard deviation.

Table 2: Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in normal thyroid (NT) and thyroid benign nodules (TBN) (nodular tissue)

Element	Thyroid tissue				Ratio
	NT	TBN nodular	Student's t-test, *p*≤	U-test, *p*	TBN nodular/NT
Ag	0.0133±0.0013	0.192±0.028	**0.000001**	**≤0.01**	14.4
Al	10.5±1.8	27.3±4.2	**0.00059**	**≤0.01**	2.60
B	0.476±0.058	4.65±2.7	0.133	>0.05	9.77
Be	0.00052±0.00008	0.00090±0.00021	0.093	>0.05	1.73
Bi	0.0072±0.0022	0.0706±0.0160	**0.00050**	**≤0.01**	9.81
Cd	2.08±0.27	1.55±0.30	0.192	>0.05	0.75
Ce	0.0080±0.0011	0.0181±0.0030	**0.0064**	**≤0.01**	2.26
Co	0.0390±0.0031	0.0576±0.0045	**0.00093**	**≤0.01**	1.48
Cr	0.495±0.031	1.17±0.17	**0.00023**	**≤0.01**	2.36
Cs	0.0245±0.0022	0.0320±0.0090	0.423	>0.05	1.31
Er	0.000377±0.000050	0.00303±0.00100	**0.000098**	**≤0.01**	8.04
Fe	222.8±9.6	430±67	**0.0031**	**≤0.01**	1.93
Ga	0.0316±0.0021	0.0211±0.0020	**0.00038**	**≤0.01**	0.67
Hg	0.0543±0.0043	1.15±0.14	**0.000001**	**≤0.01**	21.2
La	0.00475±0.00062	0.00939±0.00200	**0.017**	**≤0.01**	1.98
Li	0.0208±0.0022	0.0295±0.0030	**0.018**	**≤0.01**	1.42
Mn	1.28±0.07	1.81±0.21	**0.022**	**≤0.01**	1.41
Mo	0.0836±0.0062	0.193±0.021	**0.000017**	**≤0.01**	2.31
Nd	0.0041±0.0004	0.0134±0.0020	**0.000020**	**≤0.01**	3.27
Ni	0.449±0.046	2.89±0.47	**0.000016**	**≤0.01**	6.44
Pb	0.233±0.033	1.31±0.41	**0.013**	**≤0.01**	5.62
Pr	0.00107±0.00011	0.00389±0.00100	**0.00019**	**≤0.01**	3.64
Rb	7.54±0.39	9.50±0.50	**0.0025**	**≤0.01**	1.26
Sb	0.0947±0.0075	0.121±0.015	0.122	>0.05	1.28
Sc	0.0268±0.0060	0.0239±0.0060	0.717	>0.05	0.89
Se	2.22±0.14	3.20±0.39	**0.020**	**≤0.01**	1.44
Sm	0.000507±0.000064	0.00171±0.00032	**0.00079**	**≤0.01**	3.37
Sn	0.0777±0.0091	0.0516±0.0070	**0.027**	**≤0.01**	0.66
Tl	0.000932±.000068	0.00190±0.00020	**0.000065**	**≤0.01**	2.04
U	0.000443±0.000059	0.00116±0.00018	**0.0036**	**≤0.01**	2.62
Y	0.00260±0.00032	0.0110±0.0030	**0.0044**	**≤0.01**	4.23
Zn	94.8±4.2	117.7±5.8	**0.0018**	**≤0.01**	1.24

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

Table 3: Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in normal thyroid (NT) and thyroid benign nodules (TBN) (adjacent tissue)

Element	Thyroid tissue				Ratio
	NT	TBN adjacent	Student's t-test, $p\leq$	U-test, p	TBN adjacent/NT
Ag	0.0133±0.0013	0.473±0.130	**0.0016**	**≤0.01**	35.6
Al	10.5±1.8	25.7±7.1	0.120	>0.05	2.45
B	0.476±0.058	1.70±0.35	0.070	>0.05	3.57
Be	0.00052±0.00008	0.00053±0.00002	0.909	>0.05	1.02
Bi	0.0072±0.0022	0.478±0.226	0.129	>0.05	66.4
Cd	2.08±0.27	2.46±0.75	0.653	>0.05	1.18
Ce	0.0080±0.0011	0.488±0.469	0.382	>0.05	61.0
Co	0.0390±0.0031	0.0733±0.0170	0.052	**≤0.05**	1.88
Cr	0.495±0.031	0.614±0.111	0.311	>0.05	1.24
Cs	0.0245±0.0022	0.0132±0.0030	**0.045**	**≤0.01**	0.54
Er	0.000377±0.000050	<0.001	-	-	-
Fe	222.8±9.6	217±24	0.836	>0.05	0.97
Ga	0.0316±0.0021	≤0.024	-	-	-
Hg	0.0543±0.0043	1.39±0.16	**0.000000003**	**≤0.01**	25.6
La	0.00475±0.00062	≤0.088	-	-	-
Li	0.0208±0.0022	0.0350±0.0110	0.288	>0.05	1.68
Mn	1.28±0.07	1.78±0.36	0.188	>0.05	1.39
Mo	0.0836±0.0062	0.127±0.031	0.247	>0.05	1.52
Nd	0.0041±0.0004	≤0.048	-	-	-
Ni	0.449±0.046	1.71±0.84	0.268	>0.05	3.81
Pb	0.233±0.033	0.83±0.35	0.190	>0.05	3.56
Pr	0.00107±0.00011	≤0.017	-	-	-
Rb	7.54±0.39	10.4±0.7	**0.0012**	**≤0.01**	1.38
Sb	0.0947±0.0075	0.131±0.030	0.251	>0.05	1.26
Sc	0.0268±0.0060	0.0058±0.0020	**0.0024**	**≤0.01**	0.22
Se	2.22±0.14	1.93±0.15	0.149	>0.05	0.87
Sm	0.000507±0.000064	≤0.0011	-	-	-
Sn	0.0777±0.0091	0.135±0.060	0.407	>0.05	1.74
Tl	0.000932±.000068	≤0.0036	-	-	-
U	0.000443±0.000059	0.00180±0.00049	0.068	>0.05	4.06
Y	0.00260±0.00032	0.0088±0.0030	0.145	>0.05	3.38
Zn	94.8±4.2	105±12	0.409	>0.05	1.11

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

DOI: doi.org/10.47363/JBBR/2022(4)144

Table 4: Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in nodular and adjacent tissue of thyroid benign nodules (TBN)

Element	Thyroid tissue				Ratio
	TBN adjacent	TBN nodular	Student's t-test $p\leq$	U-test p	Nodular/adjacent
Ag	0.473±0.130	0.192±0.028	**0.044**	**≤0.01**	0.41
Al	25.7±7.1	27.3±4.2	0.848	>0.05	1.06
B	1.70±0.35	4.65±2.7	0.287	>0.05	2.74
Be	0.00053±0.00002	0.00090±0.00021	0.083	>0.05	1.70
Bi	0.478±0.226	0.0706±0.0160	0.169	>0.05	0.15
Cd	2.46±0.75	1.55±0.30	0.316	>0.05	0.63
Ce	0.488±0.469	0.0181±0.0030	0.390	>0.05	0.037
Co	0.0733±0.0170	0.0576±0.0045	0.370	>0.05	0.79
Cr	0.614±0.111	1.17±0.17	**0.0070**	**≤0.01**	1.91
Cs	0.0132±0.0030	0.0320±0.0090	0.057	>0.05	2.42
Er	<0.001	0.00303±0.00100	-	-	-
Fe	217±24	430±67	**0.0037**	**≤0.01**	1.98
Ga	≤0.024	0.0211±0.0020	-	-	-
Hg	1.39±0.16	1.15±0.14	0.274	>0.05	0.83
La	≤0.088	0.00939±0.00200	-	-	-
Li	0.0350±0.0110	0.0295±0.0030	0.656	>0.05	0.84
Mn	1.78±0.36	1.81±0.21	0.946	>0.05	1.02
Mo	0.127±0.031	0.193±0.021	0.123	>0.05	1.52
Nd	≤0.048	0.0134±0.0020	-	-	-
Ni	1.71±0.84	2.89±0.47	0.292	>0.05	1.69
Pb	0.83±0.35	1.31±0.41	0.385	>0.05	1.58
Pr	≤0.017	0.00389±0.00100	-	-	-
Rb	10.4±0.7	9.50±0.50	0.306	>0.05	0.91
Sb	0.131±0.030	0.121±0.015	0.759	>0.05	0.92
Sc	0.0058±0.0020	0.0239±0.0060	**0.0040**	**≤0.01**	4.12
Se	1.93±0.15	3.20±0.39	**0.0033**	**≤0.01**	1.66
Sm	≤0.0011	0.00171±0.00032	-	-	-
Sn	0.135±0.060	0.0516±0.0070	0.256	>0.05	0.38
Tl	≤0.0036	0.00190±0.00020	-	-	-
U	0.00180±0.00049	0.00116±0.00018	0.290	>0.05	0.64
Y	0.0088±0.0030	0.0110±0.0030	0.594	>0.05	1.25
Zn	105±12	117.7±5.8	0.351	>0.05	1.12

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

Discussion

As was shown before [29,30,35,52-58] good agreement of the 50 TE mass fractions in CRM IAEA H-4, INCT-SBF-4, INCT-TL-1, and INCT-MPH-2 samples determined by both INAA-LLR and ICP-MS methods with the certified data of these CRMs indicates acceptable accuracy of the results obtained in the study of thyroid tissue samples presented in Tables 1–4.

The Ag, Al, Bi, Ce, Co, Cr, Er, Fe, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sc, Sm, Tl, U, Y, and Zn contents in "nodular" tissue were higher, while Ga and Sn content were lower in comparison with contents of these TEs in normal gland (Table 2). Significant differences between TEs contents of "normal" thyroid and TEs contents of thyroid tissue adjacent to nodules were found for Ag, Cs, Hg, Rb, and Sc. Mass fractions of Ag, Hg, and Rb in "adjacent" group of samples were approximately 36, 26, and 1.4 times, respectively, higher, while Sc content was almost 5 times lower than in "normal" thyroid (Table 3). In a general sense Al, B, Be, Bi, Cd, Ce, Co, Cs, Ga, Hg, Li, Mn, Mo, Ni, Pb, Rb, Sb, Sn, U, Y, and Zn contents found in the "nodular" and "adjacent" groups of thyroid tissue samples were very similar (Table 4). However, levels of Cr, Fe, Sc, and Se were lower, while content of Ag in "adjacent" group of samples was higher than in nodular tissue (Table 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 TEs in the etiology and pathogenesis of thyroid diseases. In our opinion, abnormal levels of many TEs in TBNs 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 nodules and comparison obtained results with TEs levels typical of "normal" thyroid gland may give additional useful information on the topic because this data show conditions of tissue in which TBNs were originated and developed. For example, results of this study demonsrate that contents Ag, Hg, and Rb in thyroid tissue in which TBNs were originated and developed were significantly higher the levels which are "normal" for thyroid gland.

Silver

Ag is a TE with no recognized trace metal value in the human body [61]. Food is the major intake source of Ag and this metal is authorised as a food additive (E174) in the EU [62]. Another source of Ag is contact with skin and mucosal surfaces because Ag is widely used in different applications (e.g., jewelry, wound dressings, or eye drops) [63]. Ag in metal form and inorganic Ag compounds ionize in the presence of water, body fluids or tissue exudates. The silver ion Ag+ is biologically active and readily interacts with proteins, amino acid residues, free anions and receptors on mammalian and eukaryotic cell membranes [64]. Besides such the adverse effects of chronic exposure to Ag as a permanent bluish-gray discoloration of the skin (argyria) or eyes (argyrosis), exposure to soluble Ag compounds may produce other toxic effects, including liver and kidney damage, irritation of the eyes, skin, respiratory, and intestinal tract, and changes in blood cells [65]. Experimental studies shown that Ag nanoparticles may affect thyroid hormone metabolism [66]. More detailed knowledge of the Ag toxicity can lead to a better understanding of the impact on human health, including thyroid function.

Mercury

In the general population, potential sources of Hg exposure include the inhalation of this metal vapor in the air, ingestion of contaminated foods and drinking water, and exposure to dental amalgam through dental care [67]. Hg is one of the most dangerous environmental pollutants [68]. The growing use of this metal in diverse areas of industry has resulted in a significant increase of environment contamination and episodes of human intoxication. Many experimental and occupational studies of Hg in different chemical states shown significant alterations in thyroid hormones metabolism and thyroid gland parenchyma [69,70]. Moreover, Hg was classified as certain or probable carcinogen by the International Agency for Research on Cancer [71]. For example, in Hg polluted area thyroid cancer incidence was almost 2 times higher than in adjacent control areas [72].

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 TBNs 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 [73]. The sourse of Rb increase in TBNs 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 [74]. It was found also that Rb has some function in immune responce [75] and that elevated concentration of Rb could modulate proliferative responses of the cell, as was shown for bone marrow leukocytes [76].These data partially clarify the possible role of Rb in etiology and pathogenesis of TBNs.

Iron

It is well known that Fe as TEs is involved in many very important functions and biochemical reactions of human body. Fe metabolism is therefore very carefully regulated at both a systemic and cellular level [77,78]. Under the impact of age and multiple environmental factors the Fe metabolism may become dysregulated with attendant accumulation of this metal excess in tissues and organs, including thyroid [25,26,29-35]. Most experimental and epidemiological data support the hypothesis that Fe overload is a risk factor for benign and malignant tumors [79]. This goitrogenic and oncogenic effect could be explained by an overproduction of ROS and free radicals [80]. Thus, on the one hand, the accumulated data suggest that Fe might be responsible for TBNs development. But, on the other hand, the elevated level of Fe was not found in thyroid tissue adjacent to nodules. It is well known that blood is the main pool for Fe in human body and therefore high vascularisation of nodular tissue may be the reason for Fe elevated levels in TBNs [81].

Selenium

The high level of Se content found just in the TBNs cannot be regarded as pure chance. The seleno-protein characterized as Se-dependent glutathione peroxidase (Se-GSH-Px) is involved in protecting cells from peroxidative damage. This enzyme may reduce tissue concentration of free radicals and hydroperoxides. It is particular important for the thyroid gland, because thyroidal functions involve oxidation of iodide, which is incorporated into thyreoglobulin, the precursor of the thyroid hormones. For oxidation of iodide thyroidal cells produce a specific thyroid peroxidase using of physiologically generated hydrogen-peroxide (H2O2) as a cofactor [82]. It follows that the thyroid parenchyma must be continuously exposed to a physiological generation of H2O2 and in normal conditions must be a balance between levels of Se (as Se-GSH-Px) and H2O2. The elevated level of Se was not found in thyroid tissue adjacent to nodules but in nodular tissue. Thus, it might be assumed that the elevated level of Se in TBNs tissue reflects an increase in concentration of free radicals and hydroperoxides during nodular transformation.

This study has several limitations. Firstly, analytical techniques employed in this study measure only fifty TEs 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 TBNs group was relatively small and prevented investigations of TEs contents in this group using differentials like gender, histological types of TBNs, nodules functional activity, stage of disease, and dietary habits of patients with TBNs. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on many TEs level alteration in nodular and adjacent to nodule tissue and shows the necessity to continue TEs research of TBNs.

Conclusion

In this work, TEs analysis was carried out in the tissue samples of TBNs (nodular and adjacent to nodules) using a combination of non-destructive INAA-LLR and destructive ICP-MS methods. It was shown that this combination is an adequate analytical tool for the determination of fifty TEs content in the tissue samples of human thyroid in norm and pathology, including needle-biopsy specimens. It was observed that Ag, Al, Bi, Ce, Co, Cr, Er, Fe, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sc, Sm, Tl, U, Y, and Zn contents in "nodular" tissue were higher, while Ga and Sn content were lower in comparison with contents of these TEs in normal gland. Mass fractions of Ag, Hg, and Rb in "adjacent" group of samples were approximately 36, 26, and 1.4 times, respectively, higher, while Sc content was almost 5 times lower than in "normal" thyroid. Contents of Al, B, Be, Bi, Cd, Ce, Co, Cs, Ga, Hg, Li, Mn, Mo, Ni, Pb, Rb, Sb, Sn, U, Y, and Zn contents found in the "nodular" and "adjacent" groups of thyroid tissue samples were very similar. However, levels of Cr, Fe, Sc, and Se were lower, while content of Ag in "adjacent" group of samples was higher than in nodular tissue.

Acknowledgements

The author is extremely grateful to Profs. Vtyurin BM and Medvedev VS, Medical Radiological Research Center, Obninsk, as well as to Dr. Choporov Yu, former Head of the Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples. The author is also grateful to Dr. Karandaschev V, Dr. Nosenko S, and Moskvina I, Institute of Microelectronics Technology and High Purity Materials, Chernogolovka, Russia, for their help in ICP-MS analysis.

Funding

There were no any sources of funding that have supported this work.

Conflict of Interest

The author has not declared any conflict of interests.

References

1. Ghartimagar D, Ghosh A, Shrestha MK, Thapa S, Talwar OP (2020) Histopathological Spectrum of Non-Neoplastic and Neoplastic Lesions of Thyroid: A Descriptive Cross-sectional Study. J Nepal Med Assoc 58: 856-861.
2. Hoang VT, Trinh CT (2020) A Review of the Pathology, Diagnosis and Management of Colloid Goitre. Eur Endocrinol 16: 131-135.
3. Popoveniuc G, Jonklaas J (2012) Thyroid nodules. Med Clin North Am 96: 329-349.
4. Derwahl M, Studer H (2000) Multinodular goitre: 'much more to it than simply iodine deficiency'. Baillieres Best Pract Res Clin Endocrinol Metab 14: 577-600.
5. Zaichick V (1998) Iodine excess and thyroid cancer. J Trace Elem Exp Med 11: 508-509.
6. Zaichick V, Iljina T (1998) Dietary iodine supplementation effect on the rat thyroid 131I blastomogenic action. In: Die Bedentung der Mengen- und Spurenelemente. 18. Arbeitstangung. Jena: Friedrich-Schiller-Universität 294-306.
7. Kim S, Kwon YS, Kim JY, Hong KH, Park YK (2019) Association between iodine nutrition status and thyroid disease-related hormone in Korean adults: Korean National Health and Nutrition Examination Survey VI (2013-2015). Nutrients 11: 2757.
8. Vargas-Uricoechea P, Pinzón-Fernández MV, Bastidas-Sánchez BE, Jojoa-Tobar E, Ramírez-Bejarano LE, Murillo-Palacios J (2019) Iodine status in the colombian population and the impact of universal salt iodization: a double-edged sword? J Nutr Metab 6239243.
9. Stojsavljević A, Rovčanin B, Krstić D, Borković-Mitić S, Paunović I, Diklić A, Gavrović-Jankulović M, Manojlović D. 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.
10. Fahim YA, Sharaf NE, Hasani IW, Ragab EA, Abdelhakim HK (2020) Assessment of thyroid function and oxidative stress state in foundry workers exposed to lead. J Health Pollut 10: 200903.
11. Liu M, Song J, Jiang Y, Lin Y, Peng J, et al. (2021) A case-control study on the association of mineral elements exposure and thyroid tumor and goiter. Ecotoxicol Environ Saf 208: 111615.
12. Zaichick V (2006) Medical elementology as a new scientific discipline. J Radioanal Nucl Chem; 269: 303-309.
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.
14. Beyersmann D, Hartwig A (2008) Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 82: 493-512.
15. Martinez-Zamudio R, Ha HC (2011) Environmental epigenetics in metal exposure. Epigenetics 6: 820-827.
16. Zaĭchik V, Raibukhin YuS, Melnik AD, Cherkashin VI (1970) Neutron-activation analysis in the study of the behavior of iodine in the organism. Med Radiol (Mosk) 15: 33-36.
17. Zaĭchik V, Matveenko EG, Vtiurin BM, Medvedev VS (1982) Intrathyroid iodine in the diagnosis of thyroid cancer. Vopr Onkol 28: 18-24.
18. Zaichick V, Tsyb AF, Vtyurin BM (1995) Trace elements and thyroid cancer. Analyst 120: 817-821.
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: 153-161.
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: 509-510.
21. Zaichick V, Zaichick S (1999) Energy-dispersive X-ray fluorescence of iodine in thyroid puncture biopsy specimens. J Trace Microprobe Tech 17: 219-232.
22. Zaichick V (2000) Relevance of, and potentiality for in vivo intrathyroidal iodine determination. Ann N Y Acad Sci 904: 630-632.
23. Zaichick V, Zaichick S (1997) Normal human intrathyroidal iodine. Sci Total Environ 206: 39-56.
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). London: Smith-Gordon; and Tokyo: Nishimura :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: 31-38.
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: 00028.

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: 1002.
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.
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: 555644.
31. Zaichick V, Zaichick S (2018) Effect of age on chemical element contents in female thyroid investigated by some nuclear analytical methods. MicroMedicine 6: 47-61.
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: 202-212.
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: 1-10.
34. Zaichick V, Zaichick S (2018) Association between age and twenty chemical element contents in intact thyroid of males. SM Gerontol Geriatr Res 2: 1014.
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: 1059-1070.
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: 107-115.
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: IGWHC.MS.ID.000113.
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: 000108.
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: 340-355.
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: 1-18.
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: 23-37.
42. Zaichick V (2021) Comparison between trace element contents in macro and micro follicular colloid goiter using neutron activation analysis. Journal of Clinical Research and Clinical Case Reports 2: 1-7.
43. Zaichick V, Zaichick S (2021) Determination of fifty trace element contents in macro and micro follicular colloid nodular goiter. American Journal of Biomedical Science & Research 13: 639-650.
44. Zaichick V (2021) Trace element contents in thyroid of patients with diagnosed nodular goiter investigated by instrumental neutron activation analysis. Journal of Medical Research and Health Sciences 4: 1405-1417.
45. Zaichick V (2021) Determination of fifty trace element contents in normal and goitrous thyroid using a combination of instrumental neutron activation analysis and inductively coupled plasma mass spectrometry. Metallomics Research 1: 1-19.
46. Zaichick V (2021) Comparison of trace element contents in normal and adenomatous thyroid investigated using instrumental neutron activation analysis. Saudi J Biomed Res 6: 246-255
47. Zaichick V (2021) Evaluation of fifty trace element contents in thyroid adenomas using a combination of instrumental neutron activation analysis and inductively coupled plasma mass spectrometry. Journal of Cancer and Oncology Research 2: 1-11.
48. Zaichick V (2021) Evaluation of ten trace elements in Riedel's Struma using neutron activation analysis. Mod Res Clin Canc Prev 1: 1-6.
49. Zaichick V (2021) Comparison of trace element contents in normal thyroid and thyroid with Hashimoto's thyroiditis using neutron activation analysis. World Journal of Advanced Research and Reviews 12: 503-511.
50. Zaichick V, Zaichick S (1997) Instrumental effect on the contamination of biomedical samples in the course of sampling. The Journal of Analytical Chemistry 51: 1200-1205.
51. Zaichick V, Zaichick S (1997) A search for losses of chemical elements during freeze-drying of biological materials. J Radioanal Nucl Chem 218: 249-253.
52. Zaichick S, Zaichick V (2011) The effect of age on Ag, Co, Cr, Fe, Hg, Sb, Sc, Se, and Zn contents in intact human prostate investigated by neutron activation analysis. App Radiat Isot 69: 827-833.
53. Zaichick V, Zaichick S (2014) Relations of the neutron activation analysis data to morphometric parameters in pediatric and nonhyperplastic young adult prostate glands. Advances in Biomedical Science and Engineering 1: 26-42.
54. Zaichick V, Zaichick S (2016) Variations in concentration and histological distribution of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn in nonhyperplastic prostate gland throughout adulthood. Jacobs Journal of Cell and Molecular Biology 2: 1-16.
55. Zaichick S, Zaichick V, Nosenko S, Moskvina I (2021) Mass fractions of 52 trace elements and zinc trace element content ratios in intact human prostates investigated by inductively coupled plasma mass spectrometry. Biol Trace Elem Res 149: 171-183.
56. Zaichick V, Zaichick S (2014) The distribution of 54 trace elements including zinc in pediatric and nonhyperplastic young adult prostate gland tissues. Journal of Clinical and Laboratory Investigation Updates 2: 1-15.
57. Zaichick V, Zaichick S (2016) Age-related changes in concentration and histological distribution of 54 trace elements in nonhyperplastic prostate of adults. Int Arch Urol Complic 2: 019.
58. Zaichick S, Zaichick V (2010) The effect of age and gender on 37 chemical element contents in scalp hair of healthy humans. Biol Trace Elem Res 134: 41-54.
59. Zaichick V (1995) Applications of synthetic reference materials in the medical Radiological Research Centre.

Fresenius J Anal Chem 352: 219-223.
60. Korelo AM, Zaichick V (1993) Software to optimize the multielement INAA of medical and environmental samples. In: Activation Analysis in Environment Protection. Dubna, Russia: Joint Institute for Nuclear Research 326-332.
61. Lansdown AB (2007) Critical observations on the neurotoxicity of silver. Crit Rev Toxicol 37: 237-250.
62. De Vos S, Waegeneers N, Verleysen E, Smeets K, Mast J (2020) Physico-chemical characterisation of the fraction of silver (nano)particles in pristine food additive E174 and in E174-containing confectionery. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 37: 1831-1846.
63. Hadrup N, Sharma AK, Loeschner K (2018) Toxicity of silver ions, metallic silver, and silver nanoparticle materials after in vivo dermal and mucosal surface exposure: A review. Regul Toxicol Pharmacol 98: 257-267.
64. Lansdown AB (2006) Silver in health care: antimicrobial effects and safety in use. Curr Probl Dermatol 33: 17-34.
65. Drake PL, Hazelwood KJ (2005) Exposure-related health effects of silver and silver compounds: a review. Ann Occup Hyg 49: 575-585.
66. Katarzyńska-Banasik D, Grzesiak M, Kowalik K, Sechman A (2021) Administration of silver nanoparticles affects ovarian steroidogenesis and may influence thyroid hormone metabolism in hens (Gallus domesticus). Ecotoxicol Environ Saf 208: 111427.
67. Kim S-A, Kwon YM, Kim S, Joung H (2016) Assessment of dietary mercury intake and blood mercury levels in the Korean population: Results from the Korean National Environmental Health Survey 2012–2014. Int J Environ Res Public Health 13: 877.
68. Clarkson TW, Magos L (2006) The toxicology of mercury and its chemical compounds. Crit Rev Toxicol 36: 609-662.
69. Correia MM, Chammas MC, Zavariz JD, Arata A, Martins LC, Marui S, Pereira LAA (2020) Evaluation of the effects of chronic occupational exposure to metallic mercury on the thyroid parenchyma and hormonal function. Int Arch Occup Environ Health 93: 491-502.
70. Hu O, Han X, Dong G, Yan W, Wang X, et al. (2020) Association between mercury exposure and thyroid hormones levels: A meta-analysis. Environ Res 196: 110928.
71. Järup L (2003) Hazards of heavy metal contamination. Br Med Bull 68: 167-182.
72. Malandrino P, Russo M, Ronchi A, Minoia C, Cataldo D, et al. (2016) Increased thyroid cancer incidence in a basaltic volcanic area is associated with non-anthropogenic pollution and biocontamination. Endocrine 53: 471-479.
73. Haibach H, Greer MA (1973) Effect of replacement of medium potassium by sodium, cesium or rubidium on in vitro iodide transport and iodoamino acid synthesis by rat thyroid. Proc Soc Exp Biol Med 143: 114-117.
74. York DA, Bray GA, Yukimura Y (1978) An enzymatic defect in the obese (ob/ob) mouse: Loss of thyroid-induced sodium- and potassium-dependent adenosinetriphosphatase. Proc Natl Acad Sci USA 75: 477-481.
75. Jones JM, Yeralan O, Hines G, Maher M, Roberts DW, Benson W (1990) Effects of lithium and rubidium on immune responses of rats. Toxicology Letters 52: 163-168.
76. Petrini M, Vaglini F, Carulli G, Azzarà A, Ambrogi F, Grassi B (1990) Rubidium is a possible supporting element for bone marrow leukocyte differentiation. Haematologica 75: 27-31.
77. Manz DH, Blanchette NI, Paul BT, Torti FM, Torti SV (2016) Iron and cancer: recent insights. Ann N Y Acad Sci 1368: 149-161.
78. Torti SV, Manz DH, Paul BT, Blanchette-Farra N, Torti FM (2018) Iron and cancer. Annu Rev Nutr 38: 97-125.
79. Selby JV, Friedman GD (1988) Epidemiologic evidence of an association between body iron stores and risk of cancer. Int J Cancer 41: 677-682.
80. Meneghini R (1997) Iron homeostasis, oxidative stress, and DNA damage. Free Radic Biol Med 23: 783-792.
81. Razy NHMP, Rahman WFWA, Win TT (2019) Expression of vascular endothelial growth factor and its receptors in thyroid nodular hyperplasia and papillary thyroid carcinoma: A Tertiary Health Care Centre based study. Asian Pac J Cancer Prev 20: 277-282.
82. Aaseth J, Frey H, Glattre E, Norheim G, Ringstad J, Thomassen Y (1990) Selenium concentrations in the human thyroid gland. Biol Trace Elem Res 24: 147-152.