Differences between trace elements of thyroid benign nodules and thyroid tissue adjacent to nodules investigated using neutron activation analysis

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Article Info

ISSN (online): 2582-8940
Volume: 03
Issue: 02
April-June 2022
Received: 15-05-2021;
Accepted: 02-06-2021
Page No: 57-64

Abstract
Background: Thyroid benign nodules (TBNs) are common worldwide and TBNs etiology 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.
Methods: Thyroid tissue levels of silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), iodine (I), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), and zinc (Zn) were prospectively evaluated in nodular tissue and tissue adjacent to nodules of 79 patients with TBNs. Measurements were performed using neutron activation analysis.
Results: It was observed that Ag, Co, Cr, Fe, Hg, Rb, Sc, and Zn contents in "nodular" tissue were higher, while I content was 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 31, 32, and 1.4 times, respectively, higher than in "normal" thyroid. Contents of Ag, Co, Rb, Sb, and Zn 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 contents of Hg and I in "adjacent" group of samples were higher than in nodular tissue. Level of I in "adjacent" group of samples almost equals the normal value.
Conclusions: This study provides evidence on many TEs level alteration in nodular and adjacent to nodule tissue. The little reduced content of I in nodular tissue could possibly be explored for differential diagnosis of TBNs and thyroid cancer.

Keywords: Trace elements, Thyroid, Thyroid benign nodules, Neutron activation analysis

1. 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 [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 TBNs must be considered as multifactorial. The present study was performed to find out differences in TE contents between the group of nodular tissues and tissue adjacent to nodules, as well as to clarify the role of some TE 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), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), iodine (I), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), and zinc (Zn) 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 short-lived radionuclides (INAA-SLR) and long-lived radionuclides (INAA-LLR), 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 [42-46].

2. 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

All tissue samples obtained from nodular tissue and visually “normal” tissue adjacent to nodules were divided into two portions using a titanium scalpel [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 contents of the TEs by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [49]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) sub-samples were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results.

The content of I were determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor (Branch of Karpov Institute, Obninsk). Details of used nuclear reaction, radionuclide, gamma-energies, spectrometric unit, sample preparation, and the quality control of results were presented in our earlier publications concerning the INAA-SLR of I contents in human thyroid [27, 28] and scalp hair [50].

A vertical channel of the same nuclear reactor was applied to determine the content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-LLR. Details of used nuclear reactions, radionuclides, gamma-energies, spectrometric unit, sample preparation and procedure of measurement were presented in our earlier publications concerning the INAA-LLR of TEs contents in human thyroid [29, 30], scalp hair [50], and prostate [51, 52].

A dedicated computer program for INAA-SLR and INAA-LLR mode optimization was used [53]. All thyroid samples for ChEs 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, 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 Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn content and for I content in “normal” thyroid were taken from our previous publications [42-46] and [54-58], respectively. 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.

3. 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, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn mass fraction in “normal”, “nodular”, and “adjacent” groups of thyroid tissue samples.

The ratios of means and the comparison of mean values of Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, 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.

4. Discussion

As was shown before [27-30, 50-52] good agreement of the TEs contents in CRM IAEA H-4 and and CRM IAEA HH-1 samples analyzed by instrumental neutron activation analysis 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, Co, Cr, Fe, Hg, Rb, Sc, and Zn contents in “nodular” tissue were higher, while I content was 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, Hg, and Rb. Mass fractions of Ag, Hg, and Rb in “adjacent” group of samples were approximately 31, 32, and 1.4 times, respectively, higher than in “normal” thyroid (Table 3). In a general sense Ag, Co, Rb, Sb, 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 contents of Hg and I in “adjacent” group of samples were higher than in nodular tissue (Table 4). The I content in “adjacent” group of samples almost equals the normal value (Table 3).

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.

Table 1: Some statistical parameters of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid and thyroid benign nodules (nodular and adjacent tissue)

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Normal thyroid	Ag	0.0151	0.0140	0.0016	0.0012	0.0800	0.0121	0.0017	0.0454
	Co	0.0399	0.0271	0.0030	0.0046	0.140	0.0327	0.0134	0.124
	Cr	0.539	0.272	0.032	0.130	1.30	0.477	0.158	1.08
	Fe	225	100	11	51.0	512	217	67.4	456
	Hg	0.0421	0.0358	0.0041	0.0065	0.180	0.0304	0.0091	0.150
	I	1841	1027	107	114	5061	1695	230	4232
	Rb	7.37	4.10	0.44	1.11	29.4	6.49	2.60	16.7
	Sb	0.111	0.072	0.008	0.0047	0.308	0.103	0.0117	0.280
	Sc	0.0046	0.0038	0.0008	0.0002	0.0143	0.0042	0.00035	0.0131
	Se	2.32	1.29	0.14	0.439	5.80	2.01	0.775	5.65
	Zn	97.8	42.3	4.5	8.10	221	91.7	34.8	186
Thyroid benign nodules (nodular tissue)	Ag	0.226	0.219	0.031	0.0020	0.874	0.179	0.0022	0.808
	Co	0.0615	0.0332	0.0046	0.0083	0.159	0.0579	0.0152	0.141
	Cr	0.966	0.844	0.121	0.075	3.65	0.673	0.109	2.76
	Fe	332	332	40	52.3	1407	186	59.9	1346
	Hg	0.924	0.649	0.088	0.0817	3.01	0.856	0.104	2.12
	I	992	901	103	29.0	3906	695	84.8	3629
	Rb	9.55	4.37	0.52	1.00	22.1	8.90	2.48	19.6
	Sb	0.137	0.116	0.016	0.0024	0.466	0.101	0.0112	0.423
	Sc	0.0144	0.0217	0.0030	0.0002	0.0910	0.0058	0.0002	0.0878
	Se	2.75	2.13	0.29	0.720	12.6	2.31	1.05	10.0
	Zn	117.7	50.0	5.9	47.0	278	107	48.8	256
Thyroid benign nodules (adjacent tissue)	Ag	0.474	0.662	0.130	0.021	3.31	0.282	0.0516	2.07
	Co	0.0728	0.0979	0.0170	0.0051	0.594	0.0525	0.0086	0.219
	Cr	0.575	0.618	0.108	0.0180	3.14	0.401	0.0596	2.19
	Fe	211	140	24	41.5	620	163	58.2	557
	Hg	1.36	0.96	0.17	0.014	4.68	1.21	0.268	4.25
	I	2158	1436	214	343	7912	1917	527	5441
	Rb	10.5	4.3	0.7	4.10	20.0	9.80	4.74	19.4
	Sb	0.131	0.174	0.030	0.0076	0.757	0.0759	0.0269	0.749
	Sc	0.0057	0.0147	0.0020	0.0002	0.0654	0.0002	0.0002	0.0468
	Se	1.95	0.87	0.15	0.647	4.34	1.65	0.906	3.66
	Zn	105	68	12	34.2	344	86.4	42.8	304

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 Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn mass fraction (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	0.0151±0.0016	0.226±0.031	0.000000008*	≤0.01*	15.0
Co	0.0399±0.0030	0.0615±0.0046	0.00016*	≤0.01*	1.54
Cr	0.539±0.032	0.966±0.121	0.0012*	≤0.01*	1.79
Fe	225±11	332±40	0.012*	≤0.01*	1.48
Hg	0.0421±0.0041	0.924±0.088	0.00000000001*	≤0.01*	21.9
I	1841±107	992±103	0.00000005*	≤0.01*	0.54
Rb	7.37±0.44	9.55±0.52	0.0016*	≤0.01*	1.30
Sb	0.111±0.008	0.137±0.016	0.143	>0.05	1.23
Sc	0.0046±0.0008	0.0144±0.0030	0.0054*	≤0.01*	3.13
Se	2.32±0.14	2.75±0.29	0.174	>0.05	1.19
Zn	97.8±4.5	117.7±5.9	0.0086*	≤0.01*	1.20

M-arithmetic mean, SEM – standard error of mean, * significant values.

Table 3: Differences between mean values (M±SEM) of Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn mass fraction (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*≤	U-test,*p*	TBN adjacent/NT
Ag	0.0151±0.0016	0.474±0.130	0.0016*	≤0.01*	31.4
Co	0.0399±0.0030	0.0728±0.0170	0.062	≤0.05*	1.82
Cr	0.539±0.032	0.575±0.108	0.750	>0.05	1.07
Fe	225±11	211±24	0.593	>0.05	0.94
Hg	0.0421±0.0041	1.36±0.17	0.000000005*	≤0.01*	32.3
I	1841±107	2158±214	0.188	>0.05	1.17
Rb	7.37±0.44	10.5±0.7	0.00078*	≤0.01*	1.42
Sb	0.111±0.008	0.131±0.030	0.512	>0.05	1.18
Sc	0.0046±0.0008	0.0057±0.0020	0.647	>0.05	1.24
Se	2.32±0.14	1.95±0.15	0.072	>0.05	0.84
Zn	97.8±4.5	105±12	0.592	>0.05	1.07

M-arithmetic mean, SEM – standard error of mean, * significant values.

Table 4: Differences between mean values (M±SEM) of Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn mass fraction (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*≤	U-test, *p*	Nodular/adjacent
Ag	0.474±0.130	0.226±0.031	0.074	>0.05	0.48
Co	0.0728±0.0170	0.0615±0.0046	0.522	>0.05	0.84
Cr	0.575±0.108	0.966±0.121	0.018*	≤0.01*	1.68
Fe	211±24	332±40	0.011*	≤0.01*	1.57
Hg	1.36±0.17	0.924±0.088	0.023*	≤0.01*	0.68
I	2158±214	992±103	0.000007*	≤0.01*	0.46
Rb	10.5±0.7	9.55±0.52	0.324	>0.05	0.91
Sb	0.131±0.030	0.137±0.016	0.873	>0.05	1.05
Sc	0.0057±0.0020	0.0144±0.0030	0.039*	≤0.01*	2.53
Se	1.95±0.15	2.75±0.29	0.015*	≤0.01*	1.41
Zn	105±12	117.7±5.9	0.330	>0.05	1.12

M-arithmetic mean, SEM-standard error of mean, * significant values.

4.1. Silver

Ag is a TE with no recognized trace metal value in the human body [59]. Food is the major intake source of Ag and this metal is authorised as a food additive (E174) in the EU [60]. 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) [61]. 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 [62]. 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 [63]. Experimental studies shown that Ag nanoparticles may affect thyroid hormone metabolism [64]. More detailed knowledge of the Ag toxicity can lead to a better understanding of the impact on human health, including thyroid function.

4.2. 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 [65]. Hg is one of the most dangerous environmental pollutants [66]. 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 [67, 68]. Moreover, Hg was classified as certain or probable carcinogen by the International Agency for Research on Cancer [69]. For example, in Hg polluted area thyroid cancer incidence was almost 2 times higher than in adjacent control areas [70].

4.3. 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 [71]. 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 [72]. It was found also that Rb has some function in immune responce [73] and that elevated concentration of Rb could modulate proliferative responses of the cell, as was shown for bone marrow leukocytes [74].These data partially clarify the possible role of Rb in etiology and pathogenesis of TBNs.

4.4. Iodine

To date, it was well established that iodine deficiency or excess has severe consequences on human health and associated with the presence of TBNs [5-8]. However, in present study neither reduced nor elevated levels of I in thyroid tissue adjacent to nodules in comparison with “normal” thyroid tissue were not found.

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 present study, benign nodular transformation is probably accompanied by a partial loss of tissue-specific functional features, which leads to a modest reduction in I content associated with functional characteristics of the human thyroid tissue. Little reduced level of I content in nodular tissue could possibly be explored for differential diagnosis of TBNs and thyroid cancer, because, as was found in our ealier studies, thyroid malignant trasformation is accompanied by a drastically loss of I accumulation [18, 75-77].

4.5. Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only eleven TEs (Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, 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 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.

5. Conclusion

In this work, TEs analysis was carried out in the tissue samples of TBNs using neutron activation analysis. It was shown that neutron activation analysis is an adequate analytical tool for the non-destructive determination of Ag, Co, Cr, Fe, Hg, I, Rb, Sb, Sc, Se, and Zn content in the tissue samples of human thyroid in norm and pathology, including needle-biopsy specimens. It was observed that Ag, Co, Cr, Fe, Hg, Rb, Sc, and Zn contents in “nodular” tissue were higher, while I content was 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 31, 32, and 1.4 times, respectively, higher than in “normal” thyroid. Contents of Ag, Co, Rb, Sb, and Zn 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 contents of Hg and I in “adjacent” group of samples were higher than in nodular tissue. Level of I in “adjacent” group of samples almost equals the normal value. It was supposed that the little reduced content of I in nodular tissue could possibly be explored for differential diagnosis of TBNs and thyroid cancer.

6. Acknowledgements

The author is extremely grateful to Profs. B.M. Vtyurin and V.S. Medvedev, Medical Radiological Research Center, Obninsk, as well as to Dr. Yu. Choporov, former Head of the Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples.

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