Universal Journal of Pharmaceutical Research
www.ujpronline.com
Available online at www.ujpronline.com
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)
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RESEARCH ARTICLE

DIAGNOSIS OF THYROID MALIGNANCY USING TRACE ELEMENTS OF NODULAR TISSUE DETERMINED BY X-RAY FLUORESCENCE ANALYSIS

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Article Info:

Article History:
Received: 4 February 2022
Reviewed: 9 March 2022
Accepted: 28 April 2022
Published: 15 May 2022

Cite this article:
Zaichick V. Diagnosis of thyroid malignancy using trace elements of nodular tissue determined by X-ray fluorescence analysis. Universal Journal of Pharmaceutical Research 2022; 7(2):7-15.
https://doi.org/10.22270/ujpr.v7i2.744

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Abstract

Background: Benign (TBN) and malignant (TMN) thyroid nodules is a common thyroid lesion. The differentiation of TMN often remains a clinical challenge and further improvements of TMN diagnostic accuracy are warranted. The aim of this study is to evaluate the possibilities of using differences in trace element contents (TEs) in nodular tissue to diagnose thyroid malignancies and to determine the sensitivity, specificity, and accuracy for most informative TEs in the diagnosis of TMN.
Methods: Contents of TEs such as bromine (Br), copper (Cu), iron (Fe), iodine (I), rubidium (Rb), strontium (Sr), and zinc (Zn) were prospectively evaluated in "normal" thyroid (NT) of 105 individuals as well as in nodular tissue of thyroids with TBN (79 patients) and to TMN (41 patients). Measurements were performed using energy-dispersive X-ray fluorescent analysis.
Results: It was observed that in TMN tissue the mean mass fractions of I and Zn were lower while the mean mass fraction of Rb was higher than in NT and TBN tissue. It was demonstrated that the I contents is nodular tissue is the most informative parameter for the diagnosis of thyroid malignancy. It was found that "Sensitivity", "Specificity" and "Accuracy" of TMN identification using the I level in the needle biopsy of affected thyroid tissue (87±5%, 96±2% and 94±2% respectively) were significantly higher than that made using ultrasound screening and cytological test of fine needle aspiration biopsy.
Conclusion: It was concluded that determination the I level in a needle biopsy of TNs using energy-dispersive X-ray fluorescent analysis, is a fast, reliable, and informative diagnostic tool that can be successfully used as an additional test of thyroid malignancy identification.
Keywords: Diagnosis of thyroid malignancy, energy-dispersive X-ray fluorescent analysis; normal thyroid; thyroid nodules; trace elements.

INTRODUCTION

Nodules are a common thyroid lesion, particularly in women. Depending on the method of examination and general population, thyroid nodules (TNs) have an incidence of 19-68%[1]. In clinical practice, TNs are classified into benign (TBN) and malignant (TMN), and among all TNs approximately 10% are TMN[2]. It is appropriate mention here that the incidence of TMN is increasing rapidly (about 5% each year) worldwide[2]. Surgical treatment is not always necessary for TBN whereas surgical treatment is required in TMN. Thus, differentiating TBN and TMN will have a great influence on thyroid therapy.
Ultrasound screening (USS) is widely used as the primary method for early detection and diagnosis of the TNs. However, there are many similarities in the USS characteristics of both TBN and TMN. For misdiagnosis prevention some computer-diagnosis systems based on the analysis of USS images were developed, however as usual these systems for the diagnosis of TMN showed accuracy, sensitivity, and specificity nearly 80%[2,3]. Therefore, when USS examination shows suspicious signs, an US-guided fine-needle aspiration biopsy is advised. Despite the fact thatfine needle aspiration biopsy has remained the diagnostic tool of choice for evaluation of USSsuspicious thyroid nodules, the differentiation of TMN often remains a diagnostic and clinical challenge since up to 30% of nodules are categorized as cytologically "indeterminate"[4]. Thus, to improve diagnostic accuracy of TMN, new technologies have to be developed for clinical applications. However, a recent systematic review and meta-analysis of molecular tests in the preoperative diagnosis of indeterminate TNs has shown that presently there is no

perfect biochemical, immunological, and genetic biomarkers to discriminate malignancy[5]. Therefore, further improvements of TMN diagnostic accuracy are warranted. During the last decades it was demonstrated that besides iodine deficiency and excess many other dietary, environmental, and occupational factors are associated with the TNs incidence[3,6-11]. Among these factors a disturbance of evolutionary stable input of many trace elements (TEs) in human body after the industrial revolution plays a significant role in etiology of TNs[12]. Besides iodine, many other TEs have also essential physiological role and involved in thyroid 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, thyroid adenoma, and cancer in comparison with normal thyroid and thyroid tissue adjacent to TNs was demonstrated[42-48].

The aim of this study is to evaluate the possibilities of using differences in bromine (Br), copper (Cu), iron (Fe), iodine (I), rubidium (Rb), strontium (Sr), and zinc (Zn) contents in nodular tissue, determined by a combination of non-destructive ^{109}Cd (^{109}Cd-EDXRF) and ^{241}Am radionuclide-induced energy-dispersive X-ray fluorescent analysis (^{241}Am-EDXRF), to diagnose thyroid malignancies and to evaluate the sensitivity, specificity, and accuracy for most informative TEs in the discrimination of TMN.

MATERIAL AND METHODS

Specimens and patients

Samples of the NT were obtained from randomly selected autopsy specimens of 105 deceased (European-Caucasian, mean age 44±21 years, range 2-87 years), who had died suddenly. The majority of deaths were due to trauma. All the deceased had undergone routine autopsy at the Forensic Medicine Department of City Hospital, Obninsk. A histological examination in the NT group was used to control the age norm conformity, as well as to confirm the absence of micro-nodules and latent cancer. This examination was done in the Morbid Anatomy Department of City Hospital, Obninsk

All patients suffered from TBN (n=79, mean age M±SD was 44±11 years, range 22-64 years) and from TMN (n=41, mean age M±SD was 46±15 years, range 16-75 years) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre (MRRC), Obninsk. Thick-needle puncture biopsy of suspicious nodules of the thyroid was performed for every patient, to permit morphological study of thyroid tissue at these sites and to estimate their TEs contents. In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. Histological conclusions for TBN were: 46 colloid goiter, 19 thyroid adenoma, 8 Hashimoto's thyroiditis, and 6 Riedel's Struma, whereas for TMN were: 25 papillary adenocarcinomas, 8 follicular adenocarcinomas, 7 solid carcinomas, and 1 reticulo-sarcoma. Samples of nodular tissue for ^{109}Cd-EDXRF and ^{241}Am-EDXRF analysis was taken from both biopsy and resected materials.

Ethical approval

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.

Laboratory methods

All tissue samples obtained from NT, TBN and TMN were divided into two portions using a titanium scalpel to prevent contamination by TEs of stainless steel[49]. 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[50]. To determine the contents of the TEs by comparison with known data for standard, aliquots of commercial, chemically pure compounds and synthetic reference materials were used[51]. Ten subsamples of the Certified Reference Material (CRM) IAEA H-4 (animal muscle) were analyzed to estimate the precision and accuracy of results. The CRM IAEA H-4 subsamples were prepared in the same way like the samples of dry homogenized nodular tissue. Details of the relevant facility for ^{109}Cd-EDXRF determination of Br, Cu, Fe, Rb, Sr, and Zn contents, methods of analysis and the quality control of results were presented in our earlier publications concerning the ^{109}Cd-EDXRF analysis of human thyroid and prostate tissue[25,26,47,52]. Detailed information on EDXRF determination of I contents with ^{241} Am radionuclide source, including methods of analysis and the quality control of results were presented in our earlier publication concerning the use of ^{241}Am-EDXRF analysis in human thyroid study[21].

Statistic

All samples for TEs analysis were prepared in duplicate and mean values of TEs contents were used in final calculation. Using Microsoft Office Excel software, some basic statistics, including, arithmetic mean, standard deviation of mean, standard error of mean, minimum and maximum values (range) was calculated for TEs contents in three groups of thyroid tissue (NT, TBN and TMN).The difference in the results between three groups of samples was evaluated by the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

Sensitivity, specificity, and accuracy analysis

The possibility of "malignant or non- malignant" discrimination using results obtained in the study was estimated by such characteristics as "Sensitivity", "Specificity", and "Accuracy". These characteristics were calculated as:

$$\text{Sensitivity} = \frac{CPT}{CPT + FNT} X100$$

$$\text{Specificity} = \frac{CNT}{CNT + FPT} X100$$

$$\text{Accuracy} = \frac{CNT + CNT}{CPT + FNT + CNT + FPT} X100$$

Where; CPT=correct positive test, FNT=false negative test; CNT=correct negative test, FPT=false positive test

Table 1: Basic statistical parameters of Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid (N) and in thyroid benign (TBN)and malignant (TMN) nodules.

El	NT, n=105		TBN, n=79		TMN, n=41	
	Mean±SD(SEM)	Range	Mean±SD(SEM)	Range	Mean±SD(SEM)	Range
Br	13.9±12.0(1.3)	1.4-54.4	412±682(98)	3.20-2628	139±203(36)	6.2-802
Cu	4.23±1.52(0.18)	0.50-7.50	10.2±9.2(1.7)	2.90-35.2	14.5±9.4(2.6)	4.00-32.6
Fe	222±102(11)	47.1-512	345±416(49)	52,0-2563	238±184(30)	54-893
I	1618±1041(108)	110-5150	1447±3313(373)	47.0-28000	71.6±72.5(11.6)	2.00-341
Rb	9.03±6.17(0.66)	1.80-42.9	8.77±4.49(0.53)	1.00-20.3	12.4±5.00(0.79)	4.80-27.4
Sr	4.55±3.22(0.37)	0.10-13.7	4.48±6.84(0.88)	0.42-32.0	6.25±7.83(1.63)	0.93-30.8
Zn	112±44.0(4.7)	6.10-221	112.9±51.4(6.1)	22.0-270	84.3±57.4(9.2)	36.7-277

El – element, M – arithmetic mean, SD – standard deviation, SEM – standard error of mean, Range – min and max values.

RESULTS

Table 1 depicts certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, range) of the Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction in thyroid tissue samples of three groups – NT, TBN and TMN. The ratios of means and the comparison of mean values of Br, Cu, Fe, I, Rb, Sr, and Zn mass fractions in pair of sample groups such as NT and TBN, NT and TMN, and also TBN and TMN is presented in Table 2. Figure 1 depicts individual data sets for Br, I, Rb, and Zn mass fraction in all samples of NT, TBN, and TMN group.

Table 2: Ratio of means and the difference between mean values of Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction(mg/kg, dry mass basis) in normal thyroid (NT) and in thyroid benign (TBN) and malignant (TMN) nodules.

El	TBN and NT			TMN and NT			TMN and TBN		
	Ratio TBN /NT	*p* t-test	*p* U-test	Ratio TMN/NT	*p* t-test	*p* U-test	Ratio TMN/ TBN	*p* t-test	*p* U-test
Br	29.6	**0.0002***	**≤0.01***	10.0	**0.0015***	**≤0.01***	0.34	**0.017***	**≤0.01***
Cu	6.67	**0.0018***	**≤0.01***	3.43	**0.0019***	**≤0.01***	1.42	0.176	>0.05
Fe	1.55	**0.018***	**≤0.01***	1.07	0.610	>0.05	0.69	0.069	>0.05
I	0.89	0.661	>0.05	0.044	**<0.0001***	**≤0.01***	0.049	**0.0004***	**≤0.01***
Rb	0.97	0.757	>0.05	1.37	**0.0013***	**≤0.01***	1.41	**0.0002***	**≤0.01***
Sr	0.98	0.948	>0.05	1.37	0.319	>0.05	1.40	0.348	>0.05
Zn	1.00	0.944	>0.05	0.75	**0.0086***	**≤0.01***	0.75	**0.012***	**≤0.01***

El – element, *t*-test - Student's *t*-test, U-test - Wilcoxon-Mann-Whitney *U*-test, * significant differences.

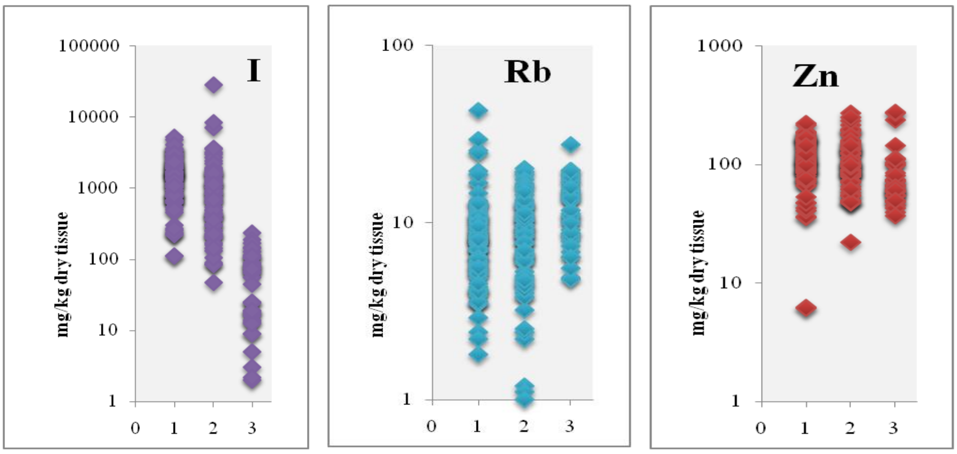

Figure 1: Individual data sets for I, Rb, and Zn mass fractions in samples of normal thyroid (1), thyroid benign nodules (2) and thyroid malignant nodules (3).

Table 3: Parameters of the sensitivity, specificity and accuracy (M±95% confidence interval) of I mass fraction for the diagnosis of TMN (an estimation is made for "TMN or NT and TBN").

Element	Upper limit for TMN (cut off)	Sensitivity %	Specificity %	Accuracy %
I	145 mg/kg dry tissue	87±5	96±2	94±2

NT - normal thyroid, TBN - thyroid benign nodules, TMN- thyroid malignant nodules.

Table 4: Reference data of I mass fractions in "normal" human thyroid published from 1990 year.

Reference	Method	n	Age, years M(Range)	Sample preparation	I, mg/kg dry tissue	
					M±SD	Range
Handl *et al.* 1990[53]	Chem	39	21-86	-	1276±664	-
Aeschimann *et al.*1994[54]	Chem	1	-	AD	2028	-
Boulyga *et al.* 1997[55]	NAA	29	-	D, A	1778±381	-
	NAA	10	-	D, A	1905±635	-
Boulyga *et al.* 1999[56]	NAA	12	-	D, A	-	800-2950
Reddy *et al.* 2002[57]	PIXE	4	-	D, Press	916±88	-
Wang *et al.* 2002[58]	-	21	Adult	-	2712±800	-
Murillo *et al.* 2005[59]	Color	5	30-43	AD	948-3356	948-3356
Hansson *et al.* 2008[60]	EDXRF	10	57-80	Intact	2400	1200-4800
Zabala *et al.* 2009[61]	SFI	50	17-60	AD	5772±2708	1676-13720
Zhu *et al*. 2010[62]	ICPMS	50	20-60	AD	2648	964-4760
Błazewicz *et al.* 2011[63]	IC	50	M=25	Fixed	601±192	624-4020
				Frozen	623±187	840 -4000
Zaichick *et al.* 2017[27]	NAA	72	2-80	Intact	1786±940	220-4205
Zaichick *et al.* 2017[28]	NAA	33	3.5-87	Intact	1956±1199	114-5061
Zaichick *et al.* 2018[31]	EDXRF, NAA	72	2-80	Intact	1786±940	220-4205
Zaichick *et al.* 2018[32]	EDXRF, NAA	33	3.5-87	Intact	1956±1199	114-5061
Zaichick *et al.* 2018[33]	NAA, ICPAES	33	3.5-87	Intact	1956±1199	114-5061
Zaichick *et al.* 2018[34]	NAA, ICPAES	72	2-80	Intact	1786±940	220-4205
Zaichick *et al.* 2018[37]	NAA	105	2-80	Intact	1841±1027	114-5061
Zaichick *et al.* 2018[64]	NAA	105	44±21	Intact	1841±1027	114-5061
Zaichick *et al.* 2018[65]	NAA	105	2-80	Intact	1841±1027	114-5061
Zaichick *et al.* 2018[66]	NAA	105	44±21	Intact	1841±1027	114-5061
Zaichick 2021[67]	NAA	105	2-87	Intact	1841±1027	114-5061
Zaichick 2021[68]	NAA	105	44±21	Intact	1841±1027	114-5061
Zaichick 2021[69]	NAA	105	2-87	Intact	1841±1027	114-5061
Zaichick 2021[70]	NAA	105	44±21	Intact	1841±1027	114-5061
Zaichick 2021[71]	NAA, ICPAES	105	2-87	Intact	1841±1027	114-5061
Zaichick 2021[72]	NAA, ICPAES	105	44±21	Intact	1841±1027	114-5061
Median of means			1841mg/kg dry tissue			
Range of means (M_{min} - M_{max}),			(601 – 5772)mg/kg dry tissue			
Ratio M_{max}/M_{min}			9.6			
All references			27			

M – arithmetic mean, SD– standard deviation of mean, Chem– chemical method, NAA– neutron activation analysis, PIXE– proton induced X-ray fluorescent emission, Color– colorimetric method, EDXRF– energy dispersive X-ray fluorescent analysis, SFI- spectrophotometric flow injection method , ICPMS – inductively coupled plasma mass spectrometry, IC- ion chromatography ,ICPAES– inductively coupled plasma atomic emission spectrometry, AD– acid digestion, D– drying at high temperature, A– ashing, AD – acid digestion.

Parameters of the sensitivity, specificity and accuracy (M±95% confidence interval) of using I mass fraction for the diagnosis of thyroid malignancy are presented in Table 3. An estimation was made from comparison individual values in TMN group with those in NT and TBN groups combined. Value of I mass fraction equals 145 mg/kg dry tissue was chosen as upper limit (cut off) for thyroid malignancy.

The comparison of obtained results with published data (from 1990 year) for I mass fraction in NT[27,28,31-34,37,53-72], TBN[54,56,57,62,63,67-80], and TMN[54,56,57,60,64-66,73,74,81-85] is shown in Table 4, Table 5, and Table 6, respectively. A number of values for TEs mass fractions were not expressed on a dry mass basis by the authors of the cited references. However, these values were calculated using published data for water (75%)[86] and ash (4.16% on dry mass basis)[87] contents in thyroid of adults.

DISCUSSION

The results of the present study of the contents of Br, Cu, Fe, I, Rb, Sr and Zn in CRM IAEA H-4 samples analyzed by EDXRF are consistent with the previously reported results before[21,25,26,47,52]. It indicates acceptable accuracy of the TEs contents in NT, TBN, and TMN groups of tissue samples presented in Table 1 to Table 3 and Figure 1. From Table 2, it is observed that in TMN tissue the mass fractions of I and Zn are significantly lower while the mass fraction of Rb is higher than in NT and TBN tissue.

Table 5: Reference data of I mass fractions in thyroid benign nodules published from 1990 year.

Reference	Method	n	Age, years M(Range)	Sample preparation	I, mg/kg dry tissue	
					M±SD	Range
Nishita *et al.* 1990[73]	NAA	14	28-71	Washed	396±74	66-1028
	NAA	7	18-74	Washed	115±40	21-344
Aeschimann *et al.*1994[54]	Chem	11	-	AD	516	92-3548
Bellisola *et al.* 1998[74]	NAA	20	17-82	Washed	660±360	560-910
	NAA	22		Washed	1140±1640	7-3810
	NAA	12		Washed	640±660	3-1840
	NAA	6		Washed	130±120	4-330
Boulyga *et al.* 1999[56]	NAA	19	-	Washed -	-	100-4050
Reddy *et al.* 2002[57]	PIXE	4	-	D, Press	888±88	-
Zhu *et al.* 2010[62]	ICPMS	50	20-60	AD	2648	964-4760
Błazewicz *et al.* 2011[63]	IC	50	M=25	Fixed	601±192	624-4020
	IC	50		Frozen	623±187	840 -4000
	IC	66	M=35	Fixed	77±14	41-104
Zaichick 2021[67]	NAA	46	30-64	Intact	1141±931	29-3715
Zaichick 2021[68]	NAA	19	41±11	Intact	961±1013	131-3906
Zaichick 2021[69]	NAA	8	40±10	Intact	951±630	83-1787
Zaichick 2021[70]	NAA	6	39±9	Intact	276±283	85-824
Zaichick 2021[71]	NAA, ICPAES	46	30-64	Intact	1141±931	29-3715
Zaichick 2021[72]	NAA, ICPAES	19	41±11	Intact	961±1013	131-3906
Zaichick 2021[75]	EDXRF, NAA	46	30-64	Intact	1144±943	29-3715
Zaichick 2021[76]	EDXRF, NAA	19	22-55	Intact	962±1013	131-3906
Zaichick 2021[77]	EDXRF, NAA	8	34-55	Intact	951±630	83-1787
Zaichick 2021[78]	NAA	6	34-50	Intact	276±283	85-824
Zaichick 2022[79]	EDXRF	79	22-64	Intact	1107±1358	47-8260
Zaichick 2022[80]	NAA, ICPAES	79	22-64	Intact	1086±1219	29-8260
Median of means				920 mg/kg dry tissue		
Range of means (M_{min} - M_{max}),				(77- 2648) mg/kg dry tissue		
Ratio M_{max}/M_{min}				34.4		
All references				20		

M – arithmetic mean, SD – standard deviation of mean, NAA – neutron activation analysis, Chem– chemical method, PIXE– proton induced X-ray fluorescent emission, ICPMS – inductively coupled plasma mass spectrometry, IC - ion chromatography, ICPAES – inductively coupled plasma atomic emission spectrometry, EDXRF– energy dispersive X-ray fluorescent analysis, AD – acid digestion.

However, as illustrated in Figure 1, I content is the most informative parameter for the diagnosis of TMN (Fig. 1). When I level of 145 mg/kg dry tissue (M+SD) was chosen as the upper limit (cut off) for TMN tissue (Fig.1), results for a "malignant or non- malignant" determination from results obtained were the following: sensitivity 87±5%, specificity 96±2%, and accuracy 94±2%. The number of people examined was taken into account for calculation of confidence intervals[88]. In other words, if I contents in a nodule biopsy sample do not exceed 145 mg/kg dry tissue, one could diagnose a thyroid malignant tumor with an accuracy of 94±2%. Using the I-test makes it possible to diagnose thyroid malignancy in 87±5% cases (sensitivity).

Thus, I content in a nodule biopsy as biomarker of TMN could become a powerful diagnostic tool. To a large extent, the resumption of the search for new methods for diagnosis of TMN was due to experience gained in a critical assessment of the limited capacity of USS examination and cytological test of fine needle aspiration biopsy[2-4]. In addition to the USS examination and morphological study of needle-biopsy of the thyroid nodules, the I-test developed in the present study seems to be very useful. Experimental conditions of the present study were approximated to the hospital conditions as closely as possible. In all cases a part of the material obtained from a puncture needle biopsy of the affected site in the thyroid was analyzed. Therefore, obtained data allowed evaluating adequately the importance of the I-test for the diagnosis of TMN. Obtained characteristics for accuracy, sensitivity, and specificity of the I-test 94%, 96%, and 87%, respectively, are significantly better than these parameters of the USS examination (nearly 80%)[2,3]. At that, the I-test gives a definite conclusion for all nodules investigated while using the morphological study of needle-biopsy up to 30% of nodules are categorized as cytologically "indeterminate"[4]. Mean values obtained for I contents in NT, TBN, and TMN agree well with median of mean values published in scientific literature for the period from 1990 up to 2022 (Table 4 to Table 6). The range of I level means reported in the literature for NT, TBN, and TMN varies greatly (Table 4 to Table 6). This discrepancy can be explained by the dependence of the I content on many factors, including age, gender, race, body mass and stage of diseases, as not all of these factors were precisely controlled in previous studies. However, in opinion of current study, the main reasons for the inter-observer discrepancy can be attributed to the accuracy of analytical techniques and sample preparation methods and the inability to take standardized samples from affected tissues.

Table 6: Reference data of I mass fractions in thyroid malignant nodules published from 1990 year.

Reference	Method	n	Age, years M (Range)	Sample preparation	I, mg/kg dry tissue	
					M±SD	Range
Nishida *et al.* 1990[73]	NAA	8	21-67	Washed	≤23±10	<DL-67
Aeschimann *et al.* 1994[54]	Chem	4	-	AD	40	16-140
Bellisola *et al.* 1998[74]	NAA	12	17-82	Washed	200±210	6 -.430
Boulyga *et al.* 1999[56]	NAA	19	-	-	-	32-900
Reddy *et al.* 2002[57]	PIXE	4	-	D, Press	<30	-
Hansson *et al.* 2008[60]	EDXRF	7	21-58	Intact	<400	-
Zaichick *et al.* 2018[64]	NAA	41	16-75	Intact	71.8±62	2-261
Zaichick *et al.* 2018[65]	EDXRF, NAA	41	46±15	Intact	71.8±62	2-261
Zaichick *et al.* 2018[66]	NAA, ICPAES	41	16-75	Intact	71.8±62	2-261
Zaichick 2022[81]	EDXRF	41	16-75	Intact	71.6±72.5	2-341
Zaichick 2022[82]	NAA	41	16-75	Intact	71.8±62	2-261
Zaichick 2022 [83]	NAA	41	16-75	Intact	71.8±62	2-261
Zaichick 2022[84]	EDXRF, NAA	41	16-75	Intact	71.8±62	2-261
Zaichick 2022[85]	NAA, ICPAES	41	16-75	Intact	71.8±62	2-261
Median of means			71.8mg/kg dry tissue			
Range of means (M_{min} - M_{max}),			(23 – 400)mg/kg dry tissue			
Ratio M_{max}/M_{min}			17.4			
All references			14			

M – arithmetic mean, SD– standard deviation of mean, NAA – neutron activation analysis, Chem– chemical method, PIXE– proton induced X-ray fluorescent emission, EDXRF– energy dispersive X-ray fluorescent analysis, ICPAES– inductively coupled plasma atomic emission spectrometry, AD– acid digestion, D– drying at high temperature.

It was insufficient quality control of results in many previous studies. In some scientific reports, tissue samples were ashed or dried at high temperature for many hours. In other cases, thyroid samples were treated with solvents (distilled water, ethanol, formalin etc). There is evidence that during ashing, drying and digestion at high temperature some quantities of I are lost as a result of this treatment[89-91].

It is well known that compared to other soft tissues, the human thyroid gland has significantly 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, malignant transformation is accompanied by a significant loss of tissue-specific functional features, which leads to a drastically reduction in I content associated with functional characteristics of the human thyroid tissue. However, it is necessary to keep in mind that biochemical, or in other words, functional changes in thyroid cells are present from the earliest development of malignancy. Thus, I depletion is an early step in the malignant proliferation process and I depletion in nodular tissue precedes the morphological transformation of cells from being histopathologically benign to malignant[12].

In current study the portable device was used for EDXRF analysis, with its ^{241}Am source for the excitation of X-ray fluorescence in the needle biopsy sample, was developed by ourselves. More powerful devices for EDXRF analysis with X-ray tubes, including “the total reflection” version (TRXRF) of the method, allow reliable determinations of I and many other TEs contents in a microprobe of a human body tissues and fluids within a few minutes[92]. EDXRF is a fully instrumental and non-destructive method because sample is investigated without requiring any pretreatment or its consumption. Moreover, it is well known that among the most modern analytical technologies, EDXRF is one of the simplest, fastest, most reliable and efficient of the available techniques for TEs determination[92]. There are many different kinds of EDXRF and TRXRF device on the market and technical improvements are frequently announced. Thus, in opinion of current study, obtaining the I level in a needle biopsy of thyroid nodule, using EDXRF, is a fast, reliable and very informative diagnostic tool that can be successfully used as an additional test for diagnoses of thyroid malignancy.

CONCLUSIONS

It can be concluded from this study that EDXRF is a suitable analytical tool for the determination of the content of Br, Cu, Fe, I, Rb, Sr, and Zn in human thyroid tissue samples, including needle biopsy material. It was observed that in TMN tissue, the mean mass fractions for I and Zn were lower while the mean mass fraction for Rb was higher than in NT and TBN tissues. Also, the iodine nodular tissue content has been proven to be the most useful parameter for diagnosing malignant tumors of the thyroid gland. It was found that the 'sensitivity', 'specificity', and 'accuracy' of TMN determination using iodine level determination by needle biopsy of affected thyroid tissue were significantly higher than those using ultrasound examination and cytological testing of fine needle biopsy. Finally, we conclude that the study of iodine level in needle biopsy of TNs, obtained using EDXRF, is a fast, reliable and informative diagnostic tool that can be successfully used as an additional test to identify thyroid malignancy.

ACKNOWLEDGEMENTS

The authoris extremely grateful to Prof. B.M. Vtyurin and Prof. V.S. Medvedev, MRRC, Obninsk, as well as to Dr. Yu. Choporov, former Head of the Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples.

AUTHOR'S CONTRIBUTION

Zaichick V: Writing original draft, review, methodology, data curation, literature survey, editing.

DATA AVAILABILITY

The datasets generated during this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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