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Selenoenzymes, Laboratory Parameters, and Trace Elements in Different Types of Thyroid Tumor

Paweł Zagrodzki · Fergus Nicol · John R. Arthur · Marian Słowiaczek · Stanisław Walas · Halina Mrowiec · Renata Wietecha-Posłuszny

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Abstract This study was performed to investigate selenoenzyme activities and trace element concentrations in thyroid tissues, with reference to other parameters routinely used to characterize thyroid function. This was to reveal relevant parameters as possible additional markers of tumor grade, clinical course, and prognosis of thyroid disorders. The tissue samples were obtained during surgical treatment (total or near total thyroidectomy) of 122 patients with different types of thyroid tumor. For most of the investigated parameters in different groups of patients, we did not find statistically significant differences. In the majority of cases, thyroid benign or malignant tumors were not accompanied by significant derangement of the gland selenoenzymes and of either intrathyroidal or plasma concentration of selenium. Nevertheless, types I and II iodothyronine deiodinases were the most promising (among selenoenzymes) targets for diagnoses and possibly therapy of thyroid tumors. Higher activities of both enzymes in cases with Graves' disease, as compared with other thyroid lesions, suggest their involvement in the pathogenesis of this condition. Patients with struna nodosa had higher levels of thyroid Zn, Cu, and Pb as compared with papillary carcinoma subjects and also a higher level of Cu than follicular

P. Zagrodzki
Department of Food Chemistry and Nutrition, Collegium Medicum Jagiellonian University, Medyczna 9, 30-688 Kraków, Poland

P. Zagrodzki (✉)
H. Niewodniczanski Institute of Nuclear Physics, Radzikowskiego 152, 31-342 Kraków, Poland
e-mail: Pawel.Zagrodzki@ifj.edu.pl

F. Nicol · J. R. Arthur
Vascular Health Division, Rowett Institute of Nutrition and Health, The University of Aberdeen, Bucksburn, Aberdeen AB21 9SB, UK

M. Słowiaczek
Third Department of General Surgery, Collegium Medicum Jagiellonian University, Prądnicka 37, 31-302 Kraków, Poland

S. Walas · H. Mrowiec · R. Wietecha-Posłuszny
Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Kraków, Poland

carcinoma cases. The above diagnostics may play a similar role to some of the general thyroid function indices, TSH, anti-TG, anti-TPO, and calcitonin, which can partially distinguish between various thyroid tumors. In conclusion, some of selenium status markers, when accompanied with general parameters, and trace elements can serve as factors with pathophysiologic relevance and be helpful in the identification of malignant disease. Multivariate statistical methods should be employed to tackle a broad array of thyroid tumor diagnostic data in a short time. Partial least squares model and other pattern recognition methods seem to be the most appropriate methods for that task. The miniaturization of all the steps of complex analytical procedure should be developed in a way to allow its completion as sensitive, robust, and efficient for use of the small quantity of material provided by fine-needle biopsy.

Keywords Selenoenzymes · Trace elements · Thyroid tumors · Thyroid hormones

Introduction

Preoperative diagnosis and novel therapeutic strategies for different types of thyroid tumors are still controversial issues. Thyroid nodules are often nonpalpable and asymptomatic and can be missed at an initial clinical presentation. Moreover, despite well-established guidelines for diagnostic management and standard treatment of thyroid tumors [1], the results of routinely used methods—fine-needle (ultrasound-monitored) aspiration cytology (FNAC) of most suspicious areas of gland or histopathological frozen section analysis—may sometimes cause difficulties in the recognition of the definite character of the tumor, either benign or malignant. The examples of such cases are follicular adenoma and carcinoma, the follicular variant of papillary carcinoma [2] or toxic multinodular goiter [3]. The most common reasons are indistinct morphological criteria or atypical focuses evolving in otherwise distinct specimens, esp. in case of very small lymph nodes. The intermediary stage of multistage transformation from prior alternative pathways of tumor etiology into a phenotype of malignant disease (characterized by expression variability of variant biochemical factors) can also hinder the rendering of the diagnosis by both clinician and pathologist. There is consent that in some circumstances, up to 10–20% of aspirated samples can be inadequate for these techniques, mainly because of a shortage of cellular material [4]. Intra-operative frozen section examination when it delivers follicular neoplasm interpretation is not helpful in decision making and defers final diagnosis until performing postoperative scrutinizing of all permanent histologic sections [5]. Such examination can be obtained only in 3–4 days postoperatively, and subsequently, secondary radical surgery can be performed not earlier than in the fifth day after first operation [6].

No essential improvement has been made by FNAC and histopathological analysis in the field of the differential diagnosis of thyroid tumors in past decades. Therefore, there is a need for more specific, biochemical, preoperative examination techniques in endocrine standards of care, strengthening the classification of human thyroid tumors. Elimination of diagnostic errors can spare patients from neck surgery or contribute to more accurate prognosis if tumor resection is really unavoidable. It is also important to diagnose patients at the earliest possible stage of disease or to estimate the probability of tumor recurrence after initial treatment in groups of low-risk patients. Pertinent, prognostic indicators serving as additional criteria for further tumor treatment regimes may restrict surgical excision, extend patients' survival, and also ensure cost savings.

Selenoproteins are involved in the protection of thyrocytes against reactive oxygen species produced during thyroid hormone synthesis. The identification of selenoenzymes in

the thyroid, belonging to three families of selenoproteins—glutathione peroxidases, deiodinases, and thioredoxin reductases—and more recently another type—SECIS-binding protein 2 (SBP2)—clarifies selenium's roles in gland function and metabolism as well as in thyroid hormone synthesis. This gives selenoprotein expression the potential to be possible sensitive indicators of major alterations of normal thyroid function. However, data concerning the selenoprotein status of normal and pathological human thyroid are relatively scarce, and the role of selenoproteins in various thyroid tumors is still not fully understood. Nonetheless, some results strongly suggest that marked disturbances in metabolism of selenium compounds can contribute to the pathogenesis of thyroid dysfunction [7, 8]. Duntas [9] has summarized up-to-date findings of selenium connection to thyroid autoimmunity and cancer.

Similarly, little is known about the predictive power of other trace element levels as indicators of the development of thyroid disorders. Few clinical studies have attempted to link trace element status with types of thyroid tumor. Our study, therefore, was performed to evaluate the relevance of determination of selected selenoproteins and trace element concentrations in thyroid tissues, with reference to other parameters routinely used to characterize thyroid function for co-ordinated procedure of the gland's diagnostics[1].

Materials and Methods

One hundred twenty-two patients, 108 women and 14 men, were studied. The age range was 17–84 years with a mean age at diagnosis of about 52±15 years; 12% of patients smoked cigarettes. The study group was ethnically homogenous. The tissue samples were obtained during surgical treatment (total or near total thyroidectomy) of patients with different types of thyroid tumor who presented to the Third Department of General Surgery, Collegium Medicum Jagiellonian University, Krakow, Poland. Thyroid fragments were immediately frozen in liquid nitrogen and kept at −20°C until use. Blood samples from patients and control subjects were drawn from the cubital vein into test tubes without anticoagulant, and after clotting, serum fraction was separated by centrifugation. For various reasons (e.g., shortage of material due to rapid and aggressive clinical course and paucity of cases), not all laboratory and statistical analysis were performed for all subjects and all types of tumors. Only the subgroup of patients with complete data sets was included in the multivariate statistical approach.

On the basis of histological follow-up routine examinations, which were available for all samples, they were allocated in eight groups as follows: struna nodosa (SN, n=38), Graves' disease, toxic nodular goiter (GD, n=9), follicular adenoma (FA, n=15), papillary carcinoma (PC, n=43), medullary carcinoma (MC, n=5), follicular carcinoma (FC, n=6), lymphoma malignant (LM, n=2), and anaplastic carcinoma (AC, n=4). Additionally, normal tissues, taken from adjacent parts of the gland, served as controls for tissue analysis (n=15). Healthy volunteers (aged 37±9 years, range 21–64 years, 41 female, 19 male) were sampled for plasma selenium indices. All volunteers were non-smokers.

[1] Preliminary results were presented at following conferences: 39th Meeting of The Polish Biochemical Society, Gdańsk, Poland, Sept. 16–20 2003; 13th International Symposium: Molecular and Physiological Aspects of Regulatory Processes of the Organism, Cracow, Poland, June 3–4, 2004; 9th Symposium of Atomic Absorption, Ustroń, Poland, Sept. 19–21 2005; 4th Conference: Chemometrics—Methods and Applications, Zakopane, Poland, Oct. 23–26, 2008.

Cytosolic (GPX1) and plasma glutathione peroxidase (GPX3), type I and type II iodothyronine deiodinases (D1 and D2), and thioredoxin reductase type I (TrxR1) were determined as the biochemical markers for selenium status of the thyroid gland. Tissues were homogenized in 0.125 M potassium phosphate buffer pH 7.4 containing 1 mM EDTA. Thereafter, homogenates were centrifuged at 20,000×*g* at 4°C for 15 min, and supernatants were used for analysis of the activities of selenoenzymes. GPX1 was evaluated with hydrogen peroxide as the substrate, as described previously [10]. D1 and D2 were determined according to the method of Sawada et al. [11], with modifications described elsewhere [10]. TrxR1 was measured spectrophotometrically using dithiobis-nitrobenzoic acid as substrate [12, 13]. Protein was determined by the Biorad dye binding method with bovine serum albumin as standard.

For trace and toxic elements analyses in thyroid tissues, the samples were digested using a microwave MARS X (CEM, Matthews, USA) system. Each thyroid sample (ca. 0.2 g) was digested in 7 mL of concentrated nitric acid (Merck) in a closed system according to a three-step procedure with the final temperature of 200°C. After cooling, nitrous oxides were removed from the digest by purging with nitrogen gas. Selenium, copper, zinc, chromium, and lead were then determined in the digest.

Copper, chromium, and lead were determined by graphite furnace atomic absorption spectrometry with the use of Varian SpectrAA Zeeman 220 spectrometer equipped with the pyrocoated tube with L'vov platform. Copper was determined (either directly in digest or after two times of dilution) at 324.8 nm. The sample was injected into the graphite furnace for analysis together with mixed matrix modifier [$Pd(NO_3)_2$/$Mg(NO_3)$ 1:2], and 2,300°C atomization temperature was applied. Chromium was determined (either directly in digests or after two times of dilution) at 257.9 nm. The sample was co-injected into the graphite furnace with the matrix modifier [$Mg(NO_3)_2$], and a 2,600°C atomization temperature was applied. Lead was determined in digests two times diluted at 283.3 nm. The sample was co-injected into the graphite furnace with the matrix modifier [$NH_4H_2PO_4$], and 1,900°C atomization temperature was applied. Zinc was determined (either directly in digest or after ten times of dilution) at 213.9 nm by flame atomic absorption spectrometry with the use of Perkin Elmer AAnalyst 300 spectrometer in manufacturer-recommended conditions. Selenium was determined by an HG-AFS method [14]. All AAS methods were validated using Pork Muscle GBW08552 Reference Material.

For some patients, the set of data was completed by determination of circulating hormones (TSH, fT4, fT3, T3, and calcitonin) and antibodies (anti-TG, and anti-TPO). Serum concentrations of TSH, anti-TPO, and calcitonin were measured using immunometric assays. The anti-TPO levels below 10 IU/mL could not be measured quantitatively. Chemiluminescent immunoassays were employed for determining serum concentrations of fT4, fT3, T3, and anti-TG. All materials used for the aforementioned analysis were from DPC Biermann Gmbh, Germany (Immulite® 2000), and all analyses were performed according to the manufacturer's instructions. The local ethical committee approved the study.

Statistical Approach

Results for each parameter were expressed as mean ± SD, although for the means coming from non-Gaussian populations, data were transformed in logarithms and retransformed after calculations.

To facilitate statistical analyses (including multivariate approaches) of three parameters (anti-TPO, anti-TG, calcitonin), the values of which were often below detection limits (DL)

or beyond the upper limit of detection, they were transformed into ordinal scale according to the following algorithm: anti-TPO, four categories: anti-TPO(1) ≤10 IU/L (below DL), anti-TPO(2) >10 IU/L, and ≤35 IU/L (below upper limit of normal range), anti-TPO(3) >35, and ≤1,000 IU/L (elevated values), anti-TPO(4) >1,000 IU/L (highly elevated values); anti-TG, three categories: anti-TG(1)≤20 IU/dL (below DL), anti-TG(2) >20 IU/dL, and ≤40 IU/dL (below upper limit of normal range), anti-TG(3) >40 IU/dL (elevated values); CT, three categories: CT(1) ≤5 pg/mL (below DL), CT(2) >5 pg/mL, and ≤125 pg/mL (normal range and moderately elevated), CT(3) >650 pg/mL (highly elevated values). For these three parameters, median values were calculated, where possible. Between groups comparison was performed using the Mann–Whitney test, which was applied only to those groups of patients with size equal or bigger than 4. Differences with $p<0.05$ were considered to be statistically significant. For the pairs of correlated parameters, obtained through previous partial least squares (PLS) approach [15], the algebraic products of corresponding weights and cosine of corresponding angle (determined by two lines connecting the origin with coordinates of respective parameters on the PLS final plot) were calculated to express the strength of bivariate associations (correlation weights). The Spearman correlation coefficients for the pairs of selenium indices were calculated in combined groups of patients. Statistical calculations were carried out using the commercially available package STATISTICA PL v.6 (StatSoft, Tulsa, USA), and the software was generously delivered by MP System Sp. z o.o. (LLC; for calculating correlation weights).

Results

The predominant thyroid tumor histological type was papillary carcinoma, amounting to 35% of our cases. The most rarely encountered cases were lymphoma malignant and anaplastic carcinoma. Male/female ratio was 1:8 and reflected the well-documented tendency of thyroid disorder prevalence in women. As the patients were being prepared for surgery, their thyroid function parameters should have been within normal ranges. However, this was not true in all patients. Among 52 patients, in whom all three indices (free thyroxine (fT4), free triiodothyronine (fT3), and thyrotropin (TSH)) were determined, only 31 were clinically euthyroid, three had values characteristic of hyperthyroidism, four were hypothyroid, six were subclinically hypothyroid (TSH > normal range, fT3, and fT4 within normal ranges), six were subclinically hyperthyroid (TSH < normal range, fT3, and fT4 within normal ranges), and two had only one parameter of these three beyond the normal range. The clinical data are summarized in Tables 1 and 2. Serum TSH discriminated between FC and two other diagnoses, GD and PC, being significantly lower in the former group. SN differed from FA and MC in respect to anti-TG, which was significantly higher in SN group.

Anti-TPO was lower in FA as compared with SN and PC. Calcitonin was very high in MC in contrast to four other groups, in which this hormone was most frequently undetectable or barely detected (Tables 1 and 2).

The descriptive statistics and a comparison of selenium indices and thyroid trace element content studied for samples categorized as different groups were detailed in Tables 3, 4, and 5. The tissues of patients with Graves' disease or toxic nodular goiter had higher activities of both D1 and D2 when compared with PC and SN (Tables 3 and 4, Fig. 1). No significant differences were found for any other selenoenzymes for various groups of patients. Patients with SN had higher levels of thyroid Zn, Cu, and Pb as compared with PC subjects and also a higher level of Cu than FC cases (Table 5).

Table 1 Summary of the Clinical Data of Patients

Parameter	Percent of cases within normal range	Cases outside normal range (no. of cases)		Differences between groups
		Below	Above	
TSH, N=109	69	15	18	FC vs. GD, p=0.045 FC vs. PC, p=0.016
T3, N=80	71	23	–	–
FT3, N=52	94	2	1	–
FT4, N=85	91	8	4	–
Anti-TG, N=76	72	–	21	SN vs. FA, p=0.024 SN vs. MC, p=0.034
Anti-TPO, N=76	64	-	26	FA vs. SN, p=0.028 FA vs. PC, p=0.047
Calcitonin, N=73	89	-	8	MC vs. SN, p=0.000 MC vs. GD, p=0.008 MC vs. FA, p=0.001 MC vs. PC, p=0.002

SN struna nodosa, *GD* Graves' disease, toxic nodular goiter, *FA* follicular adenoma, *PC* papillary carcinoma, *MC* medullary carcinoma, *FC* follicular carcinoma

In the population of patients as a whole, a significant correlation was found between D1 and D2 (R_S=0.688, p=0.000, n=82), whereas GPX1 activity correlated significantly with the TrxR1 (R_S=0.300, p=0.007, n=80) and GPX3 (R_S=0.375, p=0.001, n=76).

Previously, in the subgroup of patients consisting of 32 subjects (SN, n=8; GD, n=1; FA, n=5; PC, n=12; MC, n=1; FC, n=3; AC, n=2), for whom all the following parameters were determined (t-Zn, t-Cu, t-Cr, t-Pb, TSH, fT4, T3, anti-TPO, anti-TG, and CT (predictor parameters) and t-Se, p-Se, GPX3, and GPX1 (response parameters)), we conducted a multivariate statistical analysis PLS and pointed to some association between selenium indices and the levels of indicators of thyroid metabolism (cf. [15], see also for statistical details). Here, for pairs of correlated parameters, the correlation weights were calculated (Table 6). Weak correlations involving selenium indices were found between GPX1 and t-Pb, anti-TG(1), TSH, CT(1), CT(2) and p-Se and anti-TPO(1), anti-TPO(2), T3. No correlation was observed between t-Zn, t-Cr, and fT4 and any other parameters. PLS failed to reveal clusters of cases reflecting different diagnoses [15].

Discussion

Many specific and sensitive biochemical parameters have been tested as early markers of the modifications or abnormalities associated with the growth and proliferative potential of thyroid tumors [16–19]. Some studies, using immunohistochemical techniques with monoclonal antibodies against native human thyroid peroxidase (TPO), found qualitative and quantitative differences in the immunoreactivity of that enzyme between benign and malignant thyroid tumors [20, 21]. However, other authors did not confirm any significant alteration of TPO antigenicity in thyroid cancer tissues as compared with normal [22], and

Table 2 Thyroid Function Indices (mean ± SD, or mean ± SD based on the Log-Transformed Data; Number of Samples) in Various Groups of Patients

Group of patients	Parameters						
	TSH (μIU/mL)	T3 (ng/dL)	fT3 (pg/mL)	fT4 (ng/dL)	Anti-TPO (IU/L)	Anti-TG (IU/dL)	Calcitonin (pg/mL)
SN	0.93 (0.14–6.12)	89.3±29.4	3.06±0.65	1.59±1.36	59.4; 7, 2, 5, 7	44; 8, 2, 11	DL; 6, 14, 0
	n=33	*n*=23	*n*=19	*n*=27	*n*=21	*n*=21	*n*=20
GD	4.71±2.14	76.4±6.1	3.30±0.51	0.95±0.37	DL; 3, 0, 0, 2	DL; 4, 1, 0	DL; 3, 2, 0
	n=8	*n*=6	*n*=6	*n*=7	*n*=5	*n*=5	*n*=5
FA	1.83 (0.71–4.72)	90.1±31.2	3.20±0.56	1.20±0.28	DL; 6, 3, 0, 0	DL; 8, 0, 1	DL; 5, 4, 0
	n=12	*n*=10	*n*=5	*n*=10	*n*=9	*n*=9	*n*=9
PC	1.11 (0.25–4.93)	86.1±28.6	2.77±0.96	1.15±0.26	14.4; 11, 9, 9, 2	DL; 18, 5, 8	DL; 17, 11, 3
	n=40	*n*=30	*n*=16	*n*=29	*n*=31	*n*=31	*n*=31
MC	0.94±0.45	79.4±15.2	2.83±0.59	1.42±0.45	DL; 3, 1, 1, 0	DL; 5, 0, 0	1995; 0, 0, 5
	n=5	*n*=4	*n*=5	*n*=5	*n*=5	*n*=5	*n*=5
FC	0.50±0.38	97.7±27.3	1.96	1.25±0.62	DL; 3, 0, 0, 0	DL; 3, 0, 0	DL; 3, 0, 0
	n=5	*n*=3	*n*=1	*n*=4	*n*=3	*n*=3	*n*=3
LM	1.45	93.2	–	–	–	–	–
	n=1	*n*=1	*n*=0	*n*=0	*n*=0	*n*=0	*n*=0
AC	3.55±1.61	105.4±9.4	–	0.96±0.02	DL; 1, 1, 0	1, 0, 1	2, 0, 0
	n=3	*n*=2	*n*=0	*n*=2	*n*=2	*n*=2	*n*=2
Normal values (range)	0.4–4.0	72–170	1.5–4.1	0.8–1.9	(ND–35)	(ND–40)	≤7.4 males ≤5.4 females

For anti-TPO, anti-TG, and calcitonin, the given data represent median and the number of cases fulfilling the following conditions: anti-TPO: (1) ≤10 IU/L (below DL), (2) >0 IU/L and ≤35 IU/L (below upper limit of normal range), (3) >35 and ≤1,000 IU/L (elevated values), >1,000 IU/L (highly elevated values); anti-TG: (1) ≤20 IU/dL (below DL), (2) >20 and ≤40 IU/dL (below upper limit of normal range), (3) >40 IU/dL (elevated values); CT: (1) ≤5 pg/mL (below DL), (2) >5 and ≤125 pg/mL (normal range and moderately elevated), (3) >650 pg/mL (highly elevated values), respectively. The medians were not calculated for AC (*n*=2)

SN struna nodosa, *GD* Graves' disease, toxic nodular goiter, *FA* follicular adenoma, *PC* papillary carcinoma, *MC* medullary carcinoma, *FC* follicular carcinoma, *LM* lymphoma malignant, *AC* anaplastic carcinoma

this assay still remains controversial as a possible tool for differential diagnosis between benign and malignant tumors. In other studies, the alterations in intracellular glycosylation accompanying malignant transformation have been amply demonstrated. Particularly, cytosolic sialoglycoproteins of papillary carcinoma samples formed significantly weaker bonds with lectins than their counterparts from adenomas and non-neoplastic lesions [23]. Much research has been devoted to the influence of retinoids on the metabolism of thyrocytes, as these cells express several retinoid receptors. Retinoids exert their action as morphogens and differentiation inducing agents. They also modulate expression and activity of selenoenzymes [24–26]. Recently, gene expression profiling to diagnose benign versus malignant thyroid lesions has been developed. Although the appropriate computa-

Table 3 Selenium Indices (Arithmetic mean ± SD or mean ± SD based on the Log-Transformed Data; Number of Samples) in Various Groups of Patients

Group of patients	Parameters						
	p-Se (μmol/L)	t-Se (μg/g)	GPX3 (U/L)	GPX1 (U/mg)	TrxR1 (mU/mg)	D1 (pmol I/h/mg)	D2 (fmol I/h/mg)
SN	0.85±0.22	0.59±0.33	341.7±58.5	0.613±0.560	0.11 (0.03–0.42)	2.87±2.23	12.6±11.7
	n=27	*n*=16	*n*=27	*n*=36	*n*=30	*n*=30	*n*=30
GD	0.78±0.24	0.33 (0.10–1.15)	344.5±71.1	0.466±0.154	0.11 (0.03–0.41)	6.37±5.44	31.6±27.2
	n=5	*n*=2	*n*=5	*n*=9	*n*=8	*n*=8	*n*=8
FA	0.94±0.24	0.79±0.62	337.6±73.1	0.316 (0.104–0.958)	0.10±0.06	2.81 (0.70–11.2)	16.4 (4.7–57.3)
	n=12	*n*=9	*n*=12	*n*=14	*n*=8	*n*=8	*n*=8
PC	0.82±0.31	0.43±0.25	327.8±71.8	0.551±0.486	0.19±0.18	2.49±1.56	6.57±3.99
	n=31	*n*=15	*n*=30	*n*=34	*n*=13	*n*=13	*n*=13
MC	0.68±0.08	0.54	318.3±0.39	0.602±0.439	0.40±0.06	4.40±3.97	18.9±15.7
	n=3	*n*=1	*n*=3	*n*=4	*n*=3	*n*=3	*n*=3
FC	0.83±0.25	0.69±0.35	278.0±67.1	0.312±0.271	0.16±0.13	3.4 (1.0–11.3)	12.8 (3.9–42.6)
	n=4	*n*=4	*n*=4	*n*=6	*n*=3	*n*=3	*n*=3
LM	1.21±0.58	1.14±0.41	386.2±28.8	1.172±0.630	0.42±0.03	0.72±0.56	5.7 (1.4–24.0)
	n=2	*n*=2	*n*=2	*n*=2	*n*=2	*n*=2	*n*=2
AC	0.68±0.12	0.25±0.06	299.0±127.3	0.167±0.087	–	–	–
	n=3	*n*=3	*n*=2	*n*=3	*n*=0	*n*=0	*n*=0
Control	0.88±0.18	–	331.4±137.5	0.290 (0.093–0.909)	0.15 (0.05–0.50)	3.59±2.44	14.3±11.0
	n=59	*n*=0	*n*=59	*n*=15	*n*=13	*n*=15	*n*=15

SN struna nodosa, *GD* Graves' disease, toxic nodular goiter, *FA* follicular adenoma, *PC* papillary carcinoma, *MC* medullary carcinoma, *FC* follicular carcinoma, *LM* lymphoma malignant, *AC* anaplastic carcinoma, *p-Se* plasma selenium, *t-Se* thyroid selenium, *GPX1* thyroid cytosolic glutathione peroxidase, *GPX3* plasma glutathione peroxidase, *TrxR1* type I thioredoxin reductase, *D1* type I iodothyronine deiodinase, *D2* type II iodothyronine deiodinase

Table 4 Differences for Selenium Indices in Various Groups of Subjects

Parameter	Differences between groups
D1	SN vs. GD, p=0.026
	PC vs. GD, p=0.016
	SN vs. PC, p=0.049
D2	SN vs. GD, p=0.008
	PC vs. GD, p=0.000

SN struna nodosa, *GD* Graves' disease, toxic nodular goiter, *PC* papillary carcinoma, *D1* type I iodothyronine deiodinase, *D2* type II iodothyronine deiodinase

tional tool for analyzing data sets obtained from cDNA microarray analysis is still being questioned, the diagnostic predictor model obtained hitherto classifies the samples as benign or malignant with a very low rate of error [27]. Although there is a commercially available testing system based on pattern recognition of gene expression in the thyroid gland, it has not yet been introduced into routine diagnostic assessment of thyroid lesions. It is also not clear to what degree the changes in gene expression are concerted causative factors for tumor initiation or rather ephiphenomena acquired during tumourgenesis. However, none of the aforementioned diagnostic tools allows diagnosis and staging of all thyroid tumors on its own, and for various reasons, they have proved to be of limited value.

The present study was performed to investigate selenoenzyme activities and trace elements concentrations in thyroid tissues, with reference to other parameters routinely used to characterize thyroid function, in order to reveal relevant parameters (or clusters of such parameters) as possible additional markers of tumor grade, clinical course, and prognosis of thyroid disorders. Data on selenoenzymes in thyroid tumors and the dependency of the gland on the essential trace elements are relatively limited. Hence, we analyzed a series of pathological human thyroid tissues and also some samples of normal tissue and subsequently evaluated the selenoenzyme expression as activities.

There were no statistically significant differences for most of the investigated parameters in different groups of patients. In the majority of cases, thyroid benign or malignant tumors were not accompanied by significant derangement of the gland selenoenzymes and of either intrathyroidal or plasma concentration of selenium. Yet, it is noteworthy that the apparent differences for GPX1 or TrxR1, e.g., between LM and other groups, as well as differences for GPX3 between LM and MC and FC did not reach statistical significance, probably because of the small number of samples analyzed for these groups. Apparently, low levels of GPX1 activity in AC thyroid samples are in agreement with a decreased expression of this enzyme reported by Hasegawa et al. [28] in the same type of thyroid cancer.

Low levels of Se in serum may constitute a risk factor for thyroid cancer development since Se serum concentrations were significantly lower in cases that developed thyroid cancer in comparison to controls [29, 30]. We did not confirm such observation, and this dissociation of experimental data could be partially explained by generally low selenium status in Polish population [31]. This rationale is supported by the fact that the daily selenium intakes, calculated [31] for patients (all groups) and controls, were equal to 39.1 and 41.0 μg/day, respectively, which means that selenium status in both groups was on borderline and the difference between them was indeed very tiny.

Lack of statistically significant differences may reflect several other confounding factors such as intrinsic functional heterogeneity of thyroid tissue or differences in the stage of pathology. The attainment of euthyroidism, through the preoperative period, may also

Table 5 Thyroid Trace Element Content (mean ± SD, Number of Samples), Differences Between Groups of Patients, and Comparison with Results of Other Authors

Group of patients, number of samples	Parameters			
	t-Zn (μg/g wet mass)	t-Cu (μg/g wet mass)	t-Cr (μg/g wet mass)	t-Pb (μg/g wet mass)
SN, n=16	80.6 (34.8–187.0)	3.62±2.98	0.67 (0.14–3.12)	2.0 (0.4–10.1)
GD, n=2	309.0±140.0	7.44±2.14	1.99±1.66	1.66 (n=1)
FA, n=9	82.6±70.4	2.94±2.26	1.50±1.33	0.90 (0.15–5.42)
PC, n=15	34.2 (10.6-109.7)	2.20±1.49	0.67 (0.20-2.28)	0.58 (0.17-2.00)
MC, n=1	49.0	1.27	0.70	0.68
FC, n=4	34.2±19.2	1.52±0.48	0.26±0.13	1.04 (0.24–4.41)
LM, n=2	123.5±9.2	5.06±0.33	2.08±0.59	16.3±1.47
AC, n=3	16.3±2.76	1.47±0.51	0.27±0.08	0.43±0.22
Differences between groups of patients	SN vs. PC, p=0.027	SN vs. PC, p=0.024 SN vs. FC, p=0.022	–	SN vs. PC, p=0.043
Literature values (ref.)				
Thyroid cancer, n=21 [35]	23.1±3.6	2.65±0.31	–	–
Nodular goiter, n=41 [35]	38.3±2.2	2.82±0.62	–	–
Graves' disease, n=18 [35]	41.7±3.8	2.42±0.32	–	–
Thyroid cancer, n=5 [42]	–	–	–	0.191±0.035
Thyroid adenoma, n=13 [42]	–	–	–	0.118±0.034
Normal thyroid, [38]	39.6±4.0[a]	14.5±1.5[a]	1.6±0.2[a]	4.6±0.4[a]
Thyroid adenoma, [38]	18.2±1.8[a]	1.6±0.2[a]	9.7±1.0[a]	3.1±0.3[a]
Thyroid cancer, [38]	19.0±1.9[a]	3.4±0.3[a]	7.9±0.8[a]	4.7±0.5[a]
Normal thyroid, n=65, [36]	23±1	–	0.19±0.02	–
Thyroid cancer, n=45 [36]	19.0±1.8	–	0.25±0.05	–
Thyroid benign nodules, n=90 [36]	33.4±1.8	–	0.29±0.04	–

SN struna nodosa, *GD* Graves' disease, toxic nodular goiter, *FA* follicular adenoma, *PC* papillary carcinoma, *MC* medullary carcinoma, *FC* follicular carcinoma, *LM* lymphoma malignant, *AC* anaplastic carcinoma, *t-Zn* thyroid zinc, *t-Cu* thyroid copper, *t-Cr* thyroid chromium, *t-Pb* thyroid lead

[a] Results given for dry weight (d.w.) were converted for wet weight (w.w.) under assumption: d.w.=26.4% w.w. (19)

influence selenium status, at least in some patients. On the other hand, we did not confirm that the symptoms of either hyper- or hypothyroidism in patients with suspicious thyroid lesions reduce the likelihood of a malignancy [4], as no less than 36% of subjects, for whom all three indices (fT4, fT3, and TSH) were determined, were still either clinically or subclinically hypo- or hyperthyroid. Similarly, hyperthyroidism in patients with multinodular goiter did not protect against thyroid cancer [3].

Nevertheless, in the context of present work, D1 and D2 seem to be most promising (among selenoenzymes) targets for diagnoses and possibly therapy of thyroid tumors. Both T3-sensitive enzymes are well-characterized, but being multifactorialy regulated, they can demonstrate a broad spectrum of activities [25]. Higher activities of D1 and D2 in cases with GD, as compared to other thyroid lesions, suggest their involvement in the pathogenesis of this condition. The revealed differences are in line with evidences

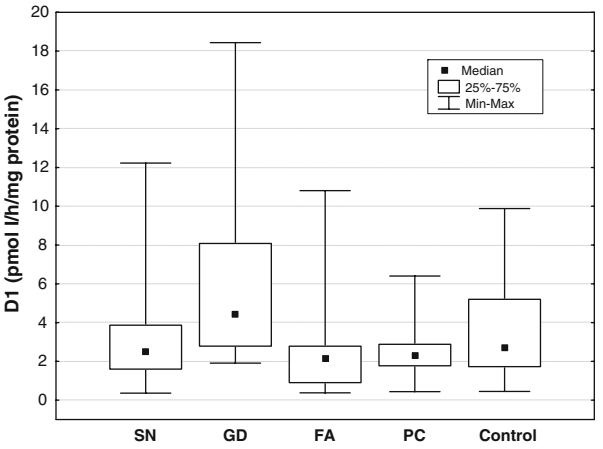

Fig. 1 The activity of iodothyronine deiodinase type I in various groups of patients

delivered by other authors [32] and support the concept of considerable metabolic alterations in Graves' disease. Whether this is a direct effect of immunoglobulin-stimulating Graves' disease on iodothyronine deiodinases or a consequence of a cascade of molecular events involved in pathogenesis of this disease remains to be established. D1 is a differentiation marker also in other thyroid carcinomas, and its activity is stimulated by retinoic acids in well-differentiated follicular thyroid carcinoma cell lines [25, 33].

The revealed correlations between D1 and D2 as well as between TrxR1 and GPX1 are in accordance with other data [34].

It is accepted that some of the general thyroid function indices (TSH, anti-TG, anti-TPO, and calcitonin) can partially distinguish between various thyroid tumors. Currently available, highly sensitive TSH determination is useful to detect hot nodules, or thyroid dysfunction, which weighs (to some extent) against the diagnosis of malignancy. Relatively higher values of TSH were observed for patients with GD (where it is a typical symptom) and in patients suffering from AC. In contrast, we proved that follicular thyroid carcinoma is rarely accompanied by overt hyperthyroidism, as FC cases showed most often normal or subnormal TSH and simultaneously fT4 levels pointing rather to subclinical hyperthyroidism. The determination of anti-TPO is a basic tool to screen for chronic thyroiditis. In the present work, the elevated levels of anti-TPO were most often found in patients with SN and PC. The

Table 6 Correlation Weights of Parameters in PLS Model

	Anti-TG(1)	t-Pb[a]	Anti-TPO(1)	Anti-TPO(2)	TSH	CT(1)[a]	CT(2)[a]	p-Se[a]
GPX1	−0.10	0.19	–	–	0.12	−0.11	0.10	–
T3	–	–	0.15	−0.16		–	–	−0.19
t-Pb[a]	−0.13	–	–	–	0.16	–	–	–
CT(1)[a]	0.10	−0.15	–	–	–	–	–	–
CT(2)[a]	−0.11	0.13	–	–	–	–	–	–
p-Se[a]	–	–	−0.13	0.18	–	–	–	–

Only weights with absolute values ≥0.1 were shown

[a] Parameters with values placed in both columns and rows

evaluation of successive parameter—serum calcitonin—can reveal unsuspected medullary thyroid carcinoma. Indeed, all patients with MC had very high calcitonin, exceeding by several times levels found in other groups.

The remarkably broad range of selenium concentrations in thyroid tissue (Table 7) suggests that factors other than the type and course of disease (occupational or environmental conditions) are more relevant determinants for the selenium concentration

Table 7 Thyroid Selenium Content in Various Groups of Subjects (mean ± SD if not Otherwise Stated)

Region	Population	*n* age (years)	Se concentration (μg/g wet mass)	Ref.
Czechoslovakia	Patients with papillary or follicular carcinomas	(8) 18–73	0.34±0.11	[37]
Czechoslovakia	Patients with adenomas	(10) 15–53	0.47±0.17	[37]
Czechoslovakia	Patients with hyperthyreosis	(10) 14–67	0.48±0.11	[37]
Czechoslovakia	Patients died consequent to various malignancies or cardiovascular diseases	(20) 76.8±6.0	0.66±0.19	[37]
Russia	Healthy adults died consequent to acute events or committed suicide	(65) 14–80	0.69±0.06[a]	[36]
Russia	Patients with different thyroid nodules	(135) 15–77	0.54±0.06[b]	[36]
			0.70±0.04[c]	
Norwey	Patients died consequent to acute illness or accidents	(45)	0.72±0.44	[43]
Austria	Patients died consequent to acute events or cardiovascular disease	(22) 25–69	0.37[d]	[44]
			0.13–0.66[e]	
Venezuela	Subjects deceased for reasons not given	–	0.505±0.051[f]–1.495±0.204[e,f]	[45]
Slovenia	Control group with no Hg exposure	(20) 33–99	0.420±0.218	[46]
Slovenia	Residents living in Hg-contaminated environment	(7) 41–76	0.474±0.165	[46]
Slovenia	Residents living near the mercury smelting plant	(2)	1.04±0.37	[46]
Canada	Adult	(1)	1.24	[47]
Poland	Patients with thyroid cancer	(21)	0.88±0.11	[35]
Poland	Patients with nodular goiter	(41)	1.49±0.34	[35]
Poland	Patients with Graves' disease	(18)	1.47±0.38	[35]
Slovenia	Mercury mine employees	(4) 33–68	2.28±2.21	[48]
			0.28–5.43[e]	
Slovenia	Retired mercury mine workers	(4) 61–68	2.59±1.9	[46]
Japan	Subjects deceased for various reasons (e.g., freezing in, acute intoxication, blood loss, suicide)	(62) 6–82	3.69±2.33	[49]
			0.78–10.8[e]	

n (number in parenthesis) number of cases

[a] Mean ± SE

[b] Mean ± SE in malignant nodules, result given for dry weight (d.w.) was converted for wet weight (w.w.) under assumption: d.w.=26.4% w.w. [36]

[c] Mean ± SE in benign nodules, result given for dry weight (d.w.) was converted for wet weight (w.w.) under assumption: d.w.=26.4% w.w. [36]

[d] Median

[e] Range

[f] Mean ± SD for different parts of single thyroid gland sample

in this gland. In particular, the highest concentrations were found in retired mercury mineworkers in Slovenia and in deceased subjects for various "accidental" reasons in Japan. Despite this fact, some studies [35–37] report a small decrease in selenium concentration in thyroid tumor. However, such changes were not always connected with the progress of malignancy of disease. There was also variation of selenium levels among our patients, but probably because of the small number of more aggressive tumor samples, they did not reach statistical significance. A similar tendency was apparent for thyroid copper concentrations [35, 38] (Table 5). The differences revealed in our study (SN vs. PC and SN vs. FC) were in accordance with results reported by others. The opposite effect for zinc thyroid content has been claimed consistently by other authors —lower concentrations reflected malignant course of disease [35, 36, 38]. In our samples, we encountered substantial scatter for zinc concentrations, with no statistical significance between groups of patients. However, the only significant difference (SN vs. PC) was in line with the above-mentioned tendency. Small sets of thyroid chromium and lead concentrations and their big scatter preclude any firm conclusion about their persistent connection with the nature of the thyroid gland. This problem needs further systematic studies. It is somewhat puzzling that results reported by Reddy and co-workers [38] were consistently much higher than mean Pb concentrations obtained by other authors as well as most of the results in current work; the reasons for this remain unclear.

The results of the partial least squares approach confirmed the existence of complex interactions among selenium status and diagnostic parameters of thyroid function. It is of interest that medium levels of CT stimulated GPX1, and similarly, the positive correlation was revealed between p-Se and medium level of anti-TPO. However, the same parameters (CT and anti-TPO) at the lowest levels exerted an opposite effect. Such findings may indicate the mobilization of selenium stores in condition of unstable euthyroidism. Positive correlation between GPX1 and TSH may be connected with the stimulation of thyrocytes metabolism by this hormone, including activities of selenoenzymes responsible for antioxidant defense [7]. The positive correlation between t-Pb and GPX1 can be ascribed to the antioxidant and detoxification function of this enzyme. Negative correlation between p-Se and T3 was somewhat unexpected as judged from the results of animal experiments, but associations can occur in humans [39–41].

The PLS approach indicates that there seems to be synergistic as well antagonistic interactions between the parameters investigated at different levels of endocrine regulation. As the PLS model explained less than 40% of variability in predictor and response parameters (cf. [15]), this implies that the assessment procedures and the set of parameters were not fully adequate, and other conditions contributing to the changes of selenoenzymes activities (and possibly more closely associated with the development of thyroid tumors) are required.

Having analyzed a high number of tumors, we may conclude that at least some of selenium status markers, when accompanied with general parameters, can serve as factors with pathophysiologic relevance and be helpful in the identification of malignant disease. Nevertheless, they are not reliable enough to aid in definitive confirming the diagnosis of carcinoma. Bearing in mind our results and findings of other authors, it seems justified that risk factor analysis in the management of patients with suspicious thyroid tumors should incorporate most of aforementioned, biochemical approaches and their diagnostic adjuncts. Each of them can add some valuable (biochemical, clinical, or histologic) information to that obtained by others and contribute to proper and early diagnosis. This is of special importance for poorly differentiated thyroid carcinomas as cases may still be indeterminate or nondiagnostic when current, routine, and diagnostic techniques are used. According to

these considerations, novel, multivariate statistical methods should be employed to tackle a broad array of data in a short time. PLS and other pattern recognition methods, with optional features of prediction and classification, seem to be the most appealing methods for that task.

Several new, promising parameters, for example, (1) SBP2, which plays a crucial role in the incorporation of Se into the selenoproteins, has a misense mutation, which elicits abnormalities in the deiodinases and consequently thyroid dysfunction; (2) selenoprotein P—responsible for Se distribution and storage and detoxification of xenobiotics in the human body; (3) gene expression of selected selenoenzymes should be taken into consideration in future studies. For this purpose, at least from the viewpoint adopted in this paper, the miniaturization of all the steps of complex analytical procedure should be developed in a way to allow its completion as sensitive, robust, and efficient, with the small quantity of material provided by fine-needle biopsy. If this is possible, and to what extent, must be clarified in future intensive investigations.

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