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Cadmium as main endocrine disruptor in papillary thyroid carcinoma and the significance of Cd/Se ratio for thyroid tissue pathophysiology

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ABSTRACT

Background: The etiology of papillary thyroid carcinoma (PTC) is unknown and some literature data support the hypothesis that heavy metals, as endocrine disrupters, could play a major role in the pathogenesis of thyroid cancer. This study aimed to estimate the content of selected toxic and essential trace metals (Mn, Co, Ni, Cu, Zn, As, Se, Cd, Pb, Th, and U), as well as the selected ratio's (Cu/Zn and Cd/Se) in the malignant thyroid tissues according to sex, age, smoking habits, familial history of any thyroid disease, pathohistological (PH) types of PTC, tumor size, the existence of a thyroid capsular invasion, intrathyroid tumor dissemination, retrosternal thyroid growth, and TNM progress of PTC.
Methods: The study included 66 patients with PTC (women/men ratio = 46/20, mean age: 54 ± 14 years). A comparative analysis was made by collecting the healthy thyroid tissues (HTTs) of the same patients, making the total number of samples 132. All trace metals were quantified by inductively coupled plasma-mass spectrometry (ICP-MS).
Results: Metals that significantly separated papillary thyroid tissues (PTTs) from the HTTs were Cd, U and Se ($p < 0.05$). The obtained negative correlation between Cd and Se in the PTTs could explain extrusion of essential Se caused by increased content of Cd. Only Cd had an influence on the retrosternal thyroid growth, while the essential metals (Mn, Co, and Zn) had an influence on thyroid capsular invasion.
Conclusion: It was found that Cd act as the main endocrine disrupter, which could highlight its role in the etiology of PTC. Considering that the Cd/Se ratio significantly separated two studied groups and had an influence on the retrosternal thyroid growth, its altered content could contribute to the better understanding of the molecular basis for pathophysiological changes in the PTC.

1. Introduction

Normal thyroid function depends on the presence of trace elements, which have a role in the synthesis and metabolism of thyroid hormones [1]. High level of trace metals is particularly dangerous for the thyroid homeostasis, as they tend to accumulate in the thyroid gland. Some literature data support the hypothesis that heavy metals, as the endocrine disrupters, could play a major role in the thyroid cancer (TC) etiology [2]. However, the mechanism of metal-induced carcinogenesis has a very complex character and may be involved in any stage of the cancer development (initiation, promotion, or progression) [3]. This indicates that each metal could have its own unique molecular mechanism which contributes to the development of cancer [4].

Thyroid cancer is the most frequent endocrine malignancy and it represents less than 1% of all human tumors [5,6]. It occurs two to four times more frequently in women than in men [7], with an age standardized (world population) rate of 6.10/100.000 for women and of 1.90/100.000 for men [8]. The neoplastic transformation of the follicular cells leads to the two main forms of well differentiated thyroid neoplasms: papillary thyroid carcinoma (PTC) and follicular thyroid

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carcinoma [9]. Papillary thyroid carcinoma is the most common form of well differentiated TCs, and it includes about 80% of all malignant TCs [10–12]. Most of TCs is diagnosed between the third and sixth decade of life [11]. There are more than 10 pathohistological (PH) variants of the PTC [13]. PTC generally grows slowly and often its indolent course makes the prognosis good in most patients [12]. For the adequate treatment of PTC, it is critical to establish an early diagnosis by performing neck ultrasound and fine needle aspiration biopsy, as well as to perform the most suitable surgical resection (hemithyroidectomy/thyroidectomy with or without dissection of neck lymph nodes). Postoperatively, an adjuvant radioiodine (^{131}I) therapy can be applied to shrinking micrometastases [5,9,13]. Beside local and regional cervical lymph node metastases, the PTC can also presents distant secondary deposits mainly in bones and lungs [13]. The mortality from TCs has tended to decline [5] and it is reported to be stable at approximately 0.5 cases per 100.000 persons [14].

Worldwide, the incidence of TC was increased in the last three decades, with the exception of some African and Scandinavian countries [14,15]. According to GLOBOCAN data, TCs occupies a 24th place in Serbia by frequency [8] and it gradually increases in the Serbian population.

The aim of this study was to compare the content of metals between malignant and healthy thyroid tissues, in order to find the most dominant trace metal(s) that could play a role in the etiology of PTC, as well as to examine the influence of dependent- and independent-biological variables, clinical and pathological variables on the metal's content for the first time. A further aim of the study was to examine if the metal's ratio could contribute to pathophysiological changes in the PTC.

2. Material and methods

2.1. Sample collection and gathered data

The study included 66 patients (women/men ratio = 46/20; mean age: 54 ± 14 years). The intravariability was achieved by using the healthy tissues of the same patients as a control group (self-control model). The healthy thyroid tissues (HTTs) were sampled with the greatest possible distance from the papillary thyroid tissues (PTTs) which were well demarked. The preoperative diagnosis of malignancy was confirmed after aspiration biopsies with a thin needle and further cytological evaluation, which was routinely performed in all patients with the suspicious nodes. A definitive diagnosis of PTC has been confirmed by two independent pathologists after postoperative pathohistological (PH) analysis of thyroid tissues. PH analysis of HTTs did not reveal a malignant change or any other pathological entity.

The gathered data included information on sex, age and smoking habits, familial history of any thyroid disease, tumor size obtained by ultrasonography, PH type of PTC, as well as the TNM (Tumor-lymph Node-Metastasis) classification, the existence of a thyroid capsular invasion, intrathyroid tumor dissemination, retrosternal thyroid growth (enlarged thyroid located behind the sternum). The clinical data for plasma thyroid hormones included values for triiodothyronine (T3) and thyroxine (T4), and its free form (fT3 and fT4), thyroid-stimulation hormone (TSH) and thyroglobulin (Tg). The patients with other malignant disease, patients with liver or kidney disease, as well as the patients on the drug therapy were excluded from this investigation. The study was approved by the Ethics Committee of Clinical Center in Belgrade, Serbia (Ethical license number: 1575/7). All patients voluntarily participated in this study and the informed consent was obtained from the each patient.

2.2. Chemicals and instrumentation

All used chemicals were of analytical grade and were supplied by Merck (Darmstadt, Germany). Concentrated nitric acid was additionally purified by double distillation. Ultrapure water with a resistance of 18.2 MΩ was obtained from a Milli Q plus system (Merck, Darmstadt, Germany). In order to prevent contamination, plastic and glass materials were immersed in a 10% nitric acid for at least 12 h and rinsed with ultrapure water before use.

For the sample preparation, a microwave oven (ETHOS 1 Advanced Microwave Digestion System, Milestone, Italy) was used. The quantification of elements was carried out by inductively coupled plasma-quadrupole-mass spectrometry, ICP-MS (iCAP Q, Thermo Scientific, UK). The pure argon gas (99.999%, Messer, Pančevo, Serbia) was used for plasma formation and the nebulization of liquid samples. The collision cell of ICP-MS was filled with pure helium (99.999%) also supplied by Messer.

A certified stock standard solution (VHG, Manchester, UK) concentration of 10 mg/L was used to prepare intermediate standard solutions. An internal standard solution (100 μg/mL Li, Sc; 20 μg/mL Bi, Ga, In, Tb, Y) was supplied by VHG, Manchester, UK. Instrument parameters of the ICP-MS were optimized using an iCAP Q tuning solution B containing 1 μg/L of Ba, Bi, Ce, Co, In, Li, and U in a 2% nitric acid (Thermo Scientific, UK). For the analytical quality assurance, standard reference material (SRM) of bovine liver (1577c, NIST, US) was employed. The results obtained by ICP-MS were additionally checked on inductively coupled plasma-optical emission spectrometry (ICP-OES, iCAP 6500 Duo Spectrometer, Thermo Scientific, UK).

All measurements on ICP-MS were performed in the kinetic energy discrimination (KED) mode by using helium. Internal standards were included to compensate matrix-induced ion signal fluctuations and the instrumental drifts. Due to the large mass range, solution of four internal standards (^{45}Sc in a concentration of 50 μg/L, and ^{89}Y, ^{159}Tb and ^{209}Bi in a concentration of 10 μg/L) was aspirated by a second channel of the peristaltic pump, allowing on-line addition to the calibration blank, standards and sample solutions.

Six calibration solutions were employed to cover the range of analyte concentrations. In the range from 1 to 300 μg/L, the linearity of calibration curves was greater than 0.998 for all elements. Based on repeated measurements (n = 3), the relative standard deviation (RSD) was below 5%. The obtained recovery values in the SRM were in the range from 88.6% to 105.7%.

2.3. Microwave sample preparation

Approximately 0.5 g of thyroid tissue was placed into microwave vessel and the exact mass was weighed. All samples were decomposed in a microwave oven at 180 °C, in a mixture of concentrated HNO_3 and concentrated H_2O_2 (4:1 v/v) by applying the following temperature program: 10 min warm-up to 180 °C and heating for 15 min. After cooling, decomposed samples were quantitatively transferred into the volumetric flasks and diluted with ultrapure water to 25 mL. The SRM was first reconstructed according to manufacturer recommendation and further processed in the same way as described for the clinical samples.

2.4. Statistical analysis

Descriptive statistics, Kruskal–Wallis one-way analysis of variance by rank test, and linear discriminant analysis (LDA) have been performed by the demo version of the NCSS statistical software (www.ncss.com). The D'Agostino-Pearson Omnibus test, Mann Whitney U-test and Spearman's Rho correlation test were performed at a significance level of $p = 0.05$ by using an SPSS statistical software (IBM SPSS Statistics 20). Principal component analysis (PCA) has been carried out by means of PLS ToolBox, v.6.2.1, for MATLAB 7.12.0 (R2011a). PCA was carried out as an exploratory data analysis by using singular value decomposition (SVD) algorithm and a 0.95 confidence level for Q and T^2 Hotelling limits for outliers.

Table 1
Clinicopathological parameters of patients with PTC.

Parameter	Ratio (yes/no)	Parameter	Values
Smoking habits	14/52	fT4 (pg/mL)	14.80 ± 3.34[a]
Heredity	18/48	fT3 (pg/mL)	4.80 ± 0.64[a]
Capsular invasion	20/46	T4 (nmol/L)	108.5 ± 25.22[a]
Retrosternal thyroid growth	7/59	T3 (nmol/L)	1.72 ± 0.46[b]
Tumor dissemination	21/45	TSH (μIU/mL)	1.18 ± 1.73[b]
		Tg (ng/mL)	92.30 ± 320.71[b]
		Tumor size (mm)	3.21 ± 1.54[a]

[a] Results with parametric distribution are presented as mean ± stdev.
[b] Results with non-parametric distribution are presented as median ± interquartile range (IQR).

3. Results

Clinicopathological parameters of patients with the PTC are presented in Table 1. Data indicated that the most of the patients did not have smoking habits, familial history of thyroid disease, thyroid capsular invasion, intrathyroid tumor dissemination, or the retrosternal thyroid growth.

Pathological examination of malignant tissues detected four different types of PTC. The most dominant PH variant was a follicular variant (34.85%), followed by micropapillary carcinoma (31.82%), classical variant (21.21%) and solid variant (12.12%). Other PH types were not found. According to the TNM classification, 30 patients were in T1 stage of disease, and only one patient was in T4 stage of the disease. The number of patients with T2 and T3 stage of the disease was the same (n = 17). For the majority of patients (n = 44) regional lymph nodes were not assessed (Nx), because there were no preoperative findings that could suggest the existence of secondary deposits. The number of patients without regional lymph node metastasis (No) and regional lymph node metastasis (N1) stage was 13 and 9, respectively. Distant metastases (Mx) were not diagnosed in any patient with PTC.

Parameters of descriptive statistics for the metal's content in the PTT and HTT group, separately, are summarized in Table 2. The content of manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), cadmium (Cd), lead (Pb), thorium (Th) and uranium (U), together with the selected ratios (Cu/Zn and Cd/Se), are presented as the median value together with parameters of its spread, such as the range. Statistically significant difference in the content of these metals between groups was confirmed by Mann-Whitney U-test (Table 2). The distribution of the elements showed that the content of Cd, U and Cd/Se were significantly higher in the PTTs comparing to HTTs, while the content of Co, Ni and Se were significantly higher in the HTTs ($p < 0.05$).

According to the results of metal content in HTT samples, only Zn was increased in men when compared to women ($p < 0.05$). The increased content of Cd, U and Cd/Se, as well as the reduced content of Cu and was found in the HTT samples of smokers compared to non-smokers. (Table S1, Supplementary material).

The data of metal's content obtained for PTTs were additionally grouped and statistically evaluated by Mann-Whitney U-test according to sex, smoking habits and clinicopathological parameters (Table 3), in order to better understand the role of metals in the etiology of PTC. The content of Cd and Cd/Se ratio was significant for differentiation of patients, according to sex and retrosternal thyroid growth. Their contents were higher in regard to retrosternal thyroid growth and they were also higher in the malignant tissues of men compared to women. The existence of a thyroid capsular invasion had an influence on the content of essential metals (Mn, Co and Zn). Thyroid tissues with capsular invasion had a lower content of these elements. According to smoking habits and intrathyroid dissemination, there were no statistically significant differences between investigated groups ($p > 0.05$).

The Kruskal-Wallis test was used to compare the medians and variances of 11 elements, as variables, for three age groups (20–40, 40–60, and older than 60 years), four PH types, three T stage and three N stages of the disease, as a single factor. If Kruskal–Wallis test had indicated a statistically significant difference between the medians ($p = 0.05$), multiple-comparison Z-value test was performed to indicate where the differences occurred (Table S2, Supplementary material). The contents of Cd, U and Cd/Se were identified as parameters that showed a significant difference among patients of different age ($p < 0.05$). Applied test also revealed that the content of Se and U separated follicular variant from a solid variant of PTC. Manganese and Cd separated T1 group from the T2, while content of Mn was also important for separation of T2 group from the T3. Manganese, Co, Pb and Th were the main variables for differentiation between No and Nx group.

Beside univariate data analysis, PCA and LDA were performed to differentiate malignant tissues from healthy one. PCA has obtained from the initial data that included all determined metals (Fig. 1) resulted in a model with low percent of variability; first two PC's explained only 37.03% of variance in the data set. New improved PCA model was obtained from data of elements that have been found as significant in the first PCA model (Co, Ni, Se, Cd, U, and Cd/Se). PCA resulted in a three component model which explained 75.91% of the total variance. The first principal component, PC1, accounted for 32.89% of the overall data variance, the second one, PC2, for 26.14% and the third principal component, PC3, for 16.88%. Mutual projections of the factor scores and their loadings for the first and third PCs are presented in Fig. 2a. Score plots of models suggested the existence of two partially overlapped groups belonging to PTT and HTT along the PC1 direction (Fig. 2b). The most influential elements discriminating HTTs were Co, Ni and Se, while Cd and Cd/Se had the highest positive influence along PC1 as main parameters that affect the separation of malignant thyroid tissues.

LDA has been performed in order to establish more valid criteria

Table 2
Parameters of descriptive statistics obtained from metal content analysis (ng/g).

		Mn	Co	Ni	Cu	Zn	As	Se	Cd	Pb	Th	U	Cu/Zn	Cd/Se
	median	137	3.02	48	355	5760	0.89	66	58	18	0.12	0.073	0.055	0.99
PTT	min	23	0.88	11	35	830	0.07	18	3	5	0.02	0.012	0.017	0.06
	max	598	18.51	525	1656	24510	6.49	302	543	102	11.21	0.402	0.211	7.34
	IQR[b]	86	3.12	55	271	6664	0.81	46	82	13	0.14	0.040	0.04	1.32
	median	120	3.96	75	319	5896	1.23	122	33	19	0.12	0.036	0.054	0.31
HTT	min	49	1.58	15	77	1319	0.15	32	2	8	0.01	0.008	0.018	0.02
	max	466	28.00	508	2018	15233	5.78	458	340	106	8.70	0.172	0.292	2.64
	IQR[b]	55	2.66	136	207	3708	1.65	103	59	13	0.12	0.040	0.040	0.38
Mann-Whitney U-test[a]	*p*	0.332	0.03	0.011	0.694	0.822	0.162	< 0.0001	0.001	0.617	0.485	< 0.0001	0.824	< 0.0001
	H_0	Reject	Accept	Accept	Reject	Reject	Reject	Accept	Accept	Reject	Reject	Accept	Reject	Accept

[a] Differences between two sets of data is significant when *p* value is less to 0.05.
[b] Interquartile range (IQR) calculated as upper quartile (X_U) – lower quartile (X_L).

Table 3
Median values for metals in PTT samples (in ng/g) and differences between two sets of data by Mann-Whitney.

	Sex			Smoking habits			Capsular invasion			Intrathyroid dissemination			Retrosternal thyroid growth		
	Women	Men	p*	Yes	No	p*	Yes	No	p*	Yes	No	p*	Yes	No	p*
Mn	133	139	0.759	132	137	0.975	112	139	**0.045**	136	138	0.453	115	139	0.219
Co	3.01	3.03	0.439	2.79	3.07	0.772	2.15	3.37	**0.017**	2.96	3.08	0.820	1.95	3.06	0.654
Ni	45	56	0.204	48	49 ±	0.944	40	54	0.696	42	50	0.772	38	52	0.603
Cu	316	376	0.563	295	357	0.567	280	367	0.063	291	356	0.504	282	356	0.349
Zn	5612	6085	0.430	4454	5939	0.419	3913	6009	**0.046**	5922	5574	0.978	6667	5651	0.795
As	0.85	0.95	0.563	1.14	0.84	0.065	0.83	0.89	0.900	0.53	1.06	0.061	0.93	0.89	0.967
Se	66	68	0.675	65	65	0.919	63	67	0.414	68	61	0.186	77	64	0.279
Cd	47	94	**0.036**	80	57	0.384	45	63	0.329	59	57	0.700	119	51	**0.030**
Pb	18	16	0.512	18	18	0.632	19	18	0.884	15	18	0.720	18	18	0.512
Th	0.12	0.11	0.294	0.12	0.12	0.729	0.13	0.12	0.450	0.12	0.12	0.709	0.11	0.12	0.294
U	0.07	0.07	0.424	0.08	0.07	0.699	0.07	0.08	0.768	0.08	0.07	0.148	0.07	0.07	0.424
Cu/Zn	0.06	0.05	0.720	0.05	0.06	0.856	0.07	0.05	0.339	0.06	0.05	0.744	0.05	0.06	0.720
Cd/Se	0.84	1.60	**0.022**	1.24	0.88	0.262	1.00	0.99	0.606	0.86	1.05	0.945	1.53	0.90	**0.022**

* Differences between two sets of data is significant when *p* value is less to 0.05.

which could be used for separation of malignant from healthy thyroid tissues. The LDA analysis resulted in one canonical function which explained 100.0% of total variance, with the eigenvalue 0.505, showing statistical significance ($F = 10.5$, and values of Wilks' lambda parameter 0.664). Standardized canonical coefficients were used for identifying the most influencing parameters and the highest absolute value was obtained for the Cd/Se (F1 = −0.67), U (F1 = −0.54) and Se (F1 = +0.50). The application of the obtained LDA model to the validation set resulted in the following misclassification rate: 53 of 66 HTTs samples (80.30%) were correctly assigned and 50 of 66 PTTs samples (75.76%) were correctly assigned, while 16 samples were misclassified as HTTs. The obtained LDA model had a good power in distinguishing PTTs from the HTTs (Fig.2c).

4. Discussion and conclusion

Many studies reported the content of essential (Cu, Zn, and Se) and some toxic metals in the blood of patients with thyroid cancers [10,16–18]. The authors pointed out that the higher content of metals in the blood could participate in the carcinogenic process. However, it should be emphasized that the increased content of metals in the blood only reflects short-term exposure of organisms to metals and could vary with the diet, professional exposure to the heavy metals, or contaminated air and drinking water [13,19]. On the other hand, there are studies that reports on quantification of metals in the malignant thyroid gland [1,13,20–25]. However, the thyroid disease cannot be generalized as a "thyroid cancer", as it should be clarified which is an exact type of TC. The other problems are related to the lack of information on the eventual presence of other pathological states, especially the chronic liver and kidney diseases that could significantly alter metal levels.

It was shown that the content of Cd in the thyroid gland is three times higher in subjects living in areas polluted with Cd when compared to subjects residing in non-polluted areas. The carcinogenic effects of Cd on the lung, prostate and testicles were widely recognized [26]. Recent studies provide evidence that Cd could play an important role in breast, pancreatic and kidney cancers [3,27]. However, there has been an insufficient investigation of Cd effects on the thyroid gland. On the other hand, studies have shown the positive effects of Se-compounds and selenoproteins in the prevention of several cancers [28]. The chemopreventive potential of Se-compounds on cancers is related to their ability to stimulate apoptosis and to inhibit tumor migration and invasion [29]. Selenium also protects follicular cells from the death induced by toxic metals by decreasing oxidative stress [30,31]. It has been reported that reduced levels of Se result in the decreased selenoprotein activity and increased TC risk [32]. The reduced Se in the cancerous tissue could be related to the altered expression of iodothyronine deiodinase (DIO) type 1 and 2 (DIO 1 and DIO 2), as well as the glutathione peroxidase (GPx). Santos et al. [33] pointed out that Se deficiency leads to a reduction in the expression and activity of DIO 1, DIO 2 and GPx. Schmutzler et al. [34] reported that selenium binding protein 1 was significantly expressed in the HTTs, but down-regulated in the PTTs. In the recent study, Metere et al. [31] found that all PTC patients showed a reduced expression of GPx1 and thioredoxin reductase (TrxR1) in the PTTs compared to those detected in the HTTs. Also, the decreased expression of selenoprotein P in papillary tissue has been found.

Our results clearly indicate that the content of Cd was significantly higher (58 vs. 33 ng/g, $p = 0.001$) and the content of essential Se was significantly lower (66 vs. 132 ng/g, $p < 0.0001$) in PTTs when

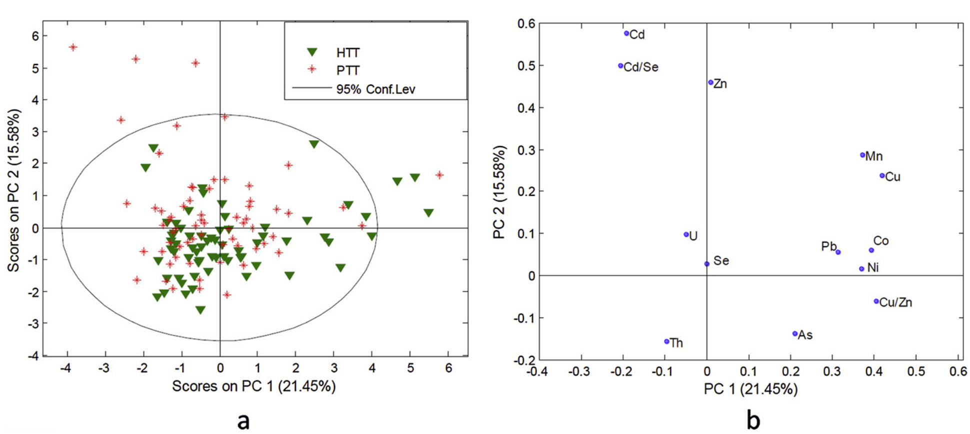

Fig. 1. Principal component analysis performed on content of all analyzed metals present in HTT and PTT samples: a) score plot as linear combinations of the data that are determined by the coefficients for each principal component, b) loading plot (the coefficients of each variable which indicate its relative weight in the principal component).

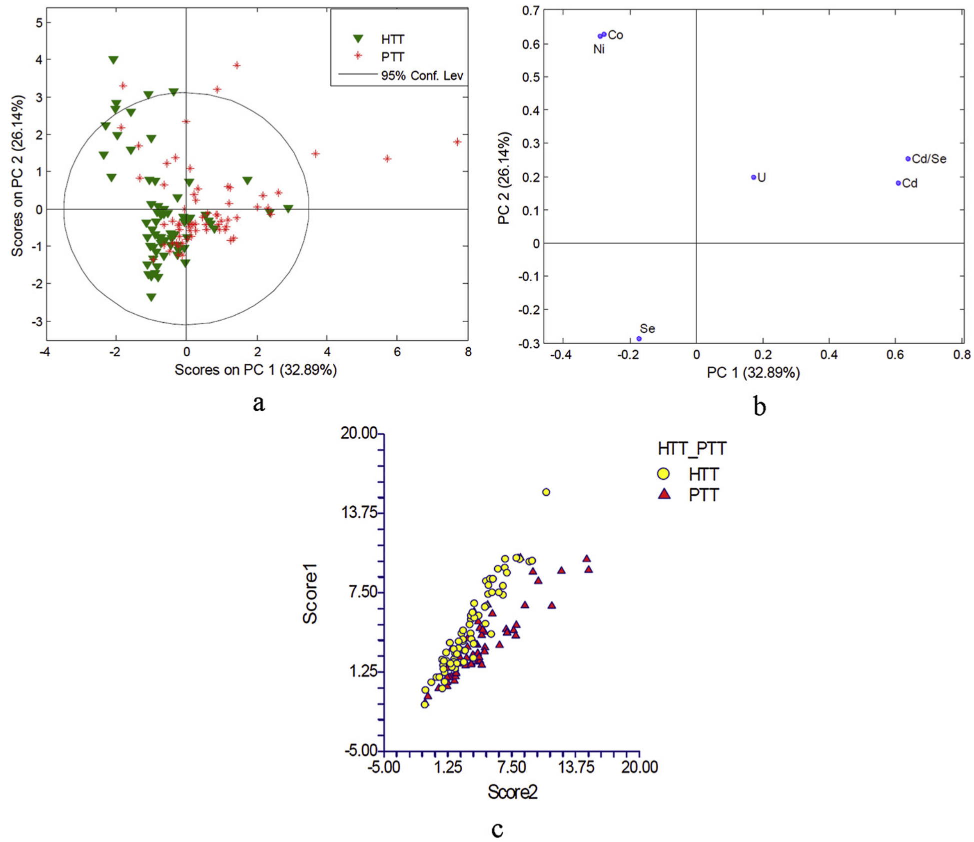

Fig. 2. Principal component analysis performed on data of elements that have been found as significant (Co, Ni, Se, Cd, U, and Cd/Se): a) score plot, b) loading plot, and c) Linear discriminant analysis score plot of first two discriminant factors (score1 and score2) obtained from analyzing data.

compared to the HTTs, respectively. It has been reported that Cd accumulated in the follicular cells can disrupt the normal functioning of mitochondria. Intraperitoneal administration of Cd-sulfate caused swelling of mitochondria and deterioration of rough-surfaced endoplasmic reticulum [27]. Kobayashi et al. [35] investigated the influences of 12 heavy metals on the human thyroid cells *in vitro*. Among them, the influence of Cd was remarkable. They also found that the toxicity of Cd decreased by the addition of high levels of selenite. These results could support negative correlation obtained for these elements in the PTTs (r_s = -0.78, at $p <$ 0.05) and could explain the antagonistic effects of lower content of Se caused by higher content of Cd in the PTTs. This type of correlation was not observed in the HTTs (r_s = 0.11, $p >$ 0.05). This interesting finding should be further confirmed in a larger number of samples.

Luca et al. [35] emphasized that the chronic exposure of hypothyroid rats to Cd and two other metals at the drinking water level of the Mt. Etna volcanic region causes an accelerated malignant transformation in the thyroid cells. Petrosino et al. [36] reported increased level of Cd in the blood of patients with TCs. Chung et al. [23] found that the chronic accumulation of Cd in the thyroid tissue is one of the aggravating factors for the progression of TC. According to our results, Cd separated T1 group from the T2, but not from the T3 group. Median values for Cd in T1, T2 and T3 groups were: 89, 41, 54 ng/g, respectively. Results of Chung et al. [23] also indicated that the content of Se was significantly higher in patients with TNM stage 3 compared to those in the stage 1. We also found that the highest content of Se was in T3 group (70 ng/g vs. 64 ng/g in T1 group), but the difference was not statistically significant. Interestingly, Mn separated the T1 group from the T2 (144 vs. 109 ng/g; $p =$ 0.047), as well as T2 from the T3 group (109 vs. 145 ng/g; $p <$ 0.05) by median values. Our results also indicated that the content of essential metals (Mn, Zn and Co) was reduced in the thyroid tissues with capsular invasion from the tissue without capsular rupture (Table 3).

We found that PTTs had significantly increased content of U when compared to HTTs (Table 2). Interestingly, uranium separated follicular variant of the solid variant (0.08 vs. 0.05 ng/g, respectively, $p =$ 0.04). The increased level of total uranium was previously reported in the whole blood samples of the adult Serbian population by Stojsavljević et al. [37].

Kucharzewski et al. [16] reported that the Cu/Zn ratio were significantly higher in the TC patients. However, the Cu/Zn ratio was proposed as a marker for various non-thyroid tumors and different pathologies [38–42]. Our results clearly indicated that the Cu/Zn did not separate PTTs from the HTTs, meaning that its significance for PTC was not of great importance.

This study had some limitations in regard to cohort size and cohort inhomogeneity (which primarily refers to the male sex), as well as in regard to the number of investigating clinical/pathological variables (such as number of patients with retrosternal thyroid growth) in order to conduct a more detailed statistical analysis. Furthermore, differences in the patient's lifestyle were not being considered, with the exception of smoking habits.

In this study it was found that the PTTs had an altered trace metal's composition when compared to the HTTs. The metals that dominantly separated PTTs from the HTTs, by their increased contents, were Cd, Se and U. Results indicated that Cd acts as the main endocrine disrupter in PTC, which could highlight its role in the unknown etiology of PTC. A strong and negative correlation between Cd and Se in the PTTs could explain the extrusion of essential Se by the increased content of Cd. Another important finding was that essential metals (Mn and Co) separated thyroid tissues with capsule invasion from those without rupture, as well as No group from the Nx. Among 11 investigated elements, only Cd had an influence on retrosternal thyroid growth. Considering that the Cd/Se ratio significantly separated two studied groups and had an influence on the retrosternal thyroid growth, its altered content could contribute to the better understanding of the molecular basis for pathophysiological changes in the PTC.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2019.06.009.

References

[1] A.K. Baltaci, T.K. Dundar, F. Aksoy, R. Mogulkoc, Changes in the serum levels of trace elements before and after the operation in thyroid cancer patients, Biol. Trace Elem. Res. 175 (2017) 57–64.
[2] A. Blazewicz, W. Dolliver, S. Sivsammye, A. Deol, R. Randhawa, G. Orlicz-Szczesna, R. Blazewicz, Determination of cadmium, cobalt, copper, iron, manganese, and zinc in thyroid glands of patients with diagnosed nodular goitre using ion chromatography, J. Chromatogr. B 878 (2010) 34–38.
[3] S.J. Mulware, Comparative trace elemental analysis in cancerous and noncancerous human tissues using PIXE, J. Biophysics 1 (2013) 1–8.
[4] S. Wang, X. Shi, Molecular mechanisms of metal toxicity and carcinogenesis, Mol. Cell. Biochem. 222 (2001) 3–9.
[5] F. Pacini, M.G. Castagna, L. Brilli, G. Pentheroudakis, Thyroid cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 23 (2012) 110–119.
[6] R.H. Grogan, E.J. Mitmaker, O.H. Clark, The evolution of biomarkers in thyroid cancer-from mass screening to a personalized biosignature, Cancers 2 (2010) 885–912.
[7] P. Malandrino, C. Scollo, I. Marturano, M. Russo, M. Tavarelli, M. Attard, P. Richuisa, M.A. Violi, G. Dardanoni, R. Vigneri, G. Pellegriti, Descriptive epidemiology of human thyroid cancer: experience from a regional registry and the „volcanic factors“, Fron. Endocrinol. 4 (2013) 1–7.
[8] J. Ferlay, E. Steliarova-Foucher, J. Lortet-Tieulent, S. Rosso, J.W.W. Coebergh, H. Comber, D. Forman, F. Bray, Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012, Eur. J. Cancer 49 (2013) 1374–1403.
[9] J.A. Fagin, S.A. Wells, Biologic and clinical perspectives on thyroid cancer, N. Engl. J. Med. 375 (2016) 1054–1067.
[10] E.O. Bursalioglu, F.A. Alkan, U.B. Barutcu, M. Demir, Y. Karabul, B. Balkan, E. Oz, O. Icelli, Prediction of electron density and trace element concentrations in human blood serum following radioiodine therapy in differentiated thyroid cancer patients, Measurement 100 (2017) 19–25.
[11] H. Katoh, K. Yamashita, T. Enomoto, M. Watanabe, Classification and general considerations of thyroid cancer, Ann. Clin. Pathol. 3 (2015) 1–9.
[12] X. Nie, Z. Tan, M. Ge, Skip metastasis in papillary thyroid carcinoma is difficult to predict in clinical practice, BMC Cancer 17 (2017) 1–8.
[13] S.B. Reddy, M.J. Charles, M.R. Kumar, B.S. Reddy, C. Anjaneyulu, G.J.N. Raju, B. Schmidbauer, K. Menhart, D. Hellwing, J. Grosse, Differentiated thyroid cancer-treatment: state of the art, Int. J. Mol. Sci. 18 (2017) 1–17.
[14] L. Devies, H.G. Welch, Increasing incidence of thyroid cancer in the United States, J. Am. Med. Assoc. 295 (2006) 2164–2167.
[15] M.A. Marcelo, P. Malandrino, J.F.M. Almeida, M.B. Martins, L.L. Cunha, N.E. Bufalo, G. Pellegriti, L.S. Ward, The influence of the environment on the development of thyroid tumors: a new appraisal, Endocr-Relat, Cancer 21 (2014) 235–254.
[16] M. Kucharzewski, J. Braziewicz, U. Majewska, S. Gozdz, Copper, zinc, and selenium in whole blood and thyroid tissue of people with various thyroid diseases, Biol. Trace Elem. Res. 93 (2003) 9–18.
[17] H. Al-Sayer, T.C. Mathew, S. Asfar, M. Khourshed, A. Al-Bader, A. Behbehani, H. Dashti, Serum changes in trace elements during thyroid cancers, Mol. Cell. Biochem. 260 (2004) 1–5.
[18] E. Przybylik-Mazurek, P. Zagrodzki, S. Kuzniarz-Rymarz, A. Hubalewska-Dydejczyk, Thyroid disorders-assessments of trace elements, clinical, and laboratory parameters, Biol. Trace Elem. Res. 141 (2011) 65–75.
[19] A. Chen, S.S. Kim, E. Chung, K.N. Dietrich, Thyroid hormones in relation to lead, mercury, and cadmium exposure in the national health and nutrition examination survey, 2007-2008, Environ. Health Perspect. 121 (2013) 181–186.
[20] U. Majewska, J. Braziewicz, D. Banas, A. Kubala-Kukus, M. Kucharzewski, J. Waler, S. Gozdz, J. Wudarczyk, Zn concentration in thyroid tissue and whole blood of women with different disease of thyroid, Biol. Trace Elem. Res. 80 (2001) 193–199.
[21] J. Gumulec, M. Masarik, V. Adam, T. Eckschlager, I. Provaznik, R. Kizek, Serum and tissue zinc in epithelial malignancies: a meta-analysis, PLoS One 9 (2014) 1–11.
[22] M. Yaman, I. Akdeniz, Sensitivity enhancement in flame atomic absorption spectrometry for determination of copper in human thyroid tissues, Anal. Sci. 20 (2004) 1363–1366.
[23] H.K. Chung, J.S. Nam, C.W. Ahn, Y.S. Lee, K.R. Kim, Some elements in thyroid tissue are associated with more advanced stage of thyroid cancer in Korean women, Biol. Trace Elem. Res. 171 (2016) 54–62.
[24] V. Zaichick, S. Zaichick, Associations between age and 50 trace element contents and relationships in intact thyroid of males, Aging Clin, Exp. Res. 30 (2018) 1059–1070.
[25] V.Y. Zaichick, A.F. Tsyb, B.M. Vtyurin, Trace elements and thyroid cancer, Analyst 120 (1995) 817–821.
[26] S.A. Jancic, B.Z. Stosic, Cadmium effects on the thyroid gland, in: G. Litwack (Ed.), Vitamins and Hormones, 2014, pp. 391–425.
[27] A. Buha, V. Matovic, B. Antonijevic, Z. Bulat, M. Curcic, E.A. Renieri, A.M. Tsatsakis, A. Schweitzer, D. Wallace, Overview of cadmium thyroid disrupting effects and mechanisms, Int. J. Mol. Sci. 19 (2018) 1–19.
[28] G. Dennert, M. Zwahlen, M. Brinkman, M. Vinceti, M.P. Zeegers, M. Horneber, Selenium for preventing cancer, Cochrane Database Syst. Rev. 11 (2011) CD005195.
[29] V. Jayaprakash, J.R. Marshall, Selenium and other antioxidants for chemoprevention of gastrointestinal cancers, Best Pract. Res. Clin. Gastroenterol. 25 (2011) 507–518.
[30] M. Selenius, A.K. Rundlof, E. Olm, A.P. Fernandes, M. Björnstedt, Selenium and the selenoprotein thioredoxin reductase in the prevention, treatment and diagnostics of cancer, Antioxid. Redox Signal. 12 (2010) 867–880.
[31] A. Metere, F. Frezzotti, C.E. Graves, M. Vergine, A. De Luca, D. Pietraforte, L. Giacomelli, A possible role for selenoprotein glutathione peroxidase (GPx1) and thioredoxin reductases (TrxR1) in thyroid cancer: our experience in thyroid surgery, Cancer Cell Int. 18 (2018) 2–9.
[32] L.H. Duntas, P.P.A. Smyth, et al., Selenoproteins and thyroid cancer, in: J. Liu (Ed.), Selenoproteins and Mimics, 2011, pp. 173–182.
[33] L.R. Santos, C. Neves, M. Melo, P. Soares, Selenium and selenoproteins in immune mediated thyroid disorders, Diagnostics 8 (2018) E70 pii:.
[34] C. Schmutzler, B. Mentrup, L. Schomburg, C. Hoang-Vu, V. Herzog, J. Kohrle, Selenoproteins of the thyroid gland: expression, localization and possible function of glutathione peroxidase 3, Bio. Chem. 388 (2007) 1053–1059.
[35] J. Kobayashi, K. Ikeda, H. Sugiyama, Cytotoxicity of heavy metals to a thyroid carcinoma cell line, J. Environ. Anal. Toxicol. 7 (2017) 1–3.
[36] E. Luca, L. Fici, A. Ronchi, F. Marandino, E.D. Rossi, M.E. Caristo, P. Malandrino, M. Russo, A. Pontecorvi, R. Vigneri, F. Moretti, Intake of boron, cadmium and molybdenium enhances rat thyroid cell transformation, J. Exp. Clin. Cancer Res. 36 (73) (2017) 1–9.
[37] V. Petrosino, G. Motta, G. Tenore, M. Coletta, A. Guarglia, D. Testa, The role of heavy metals and polychlorinated biphenyls (PCBs) in the oncogenesis of head and neck tumors and thyroid disease: a pilot study, Biometals 31 (2018) 285–295.
[38] A. Stojsavljević, S. Borković-Mitić, Lj Vujotić, D. Grujičić, M. Gavrović-Jankulović, D. Manojlović, The human biomonitoring study in Serbia: background levels for arsenic, cadmium, lead, thorium and uranium in the whole blood of adult Serbian population, Ecotox. Environ. Safety 169 (2019) 402–409.
[39] K. Zabłocka-Słowińska, S. Płaczkowskab, A. Preschaa, K. Pawełczykc, I. Porębskad, M. Kosackad, L. Pawlik-Sobeckae, H. Grajetaa, Serum and whole blood Zn, Cu and Mn profiles and their relation to redox status in lung cancer patients, J. Trace Elem. Med. Biol. 45 (2018) 78–84.
[40] M. Wacewicz, K. Socha, J. Soroczyńska, M. Niczyporuk, P. Aleksiejczuk, J. Ostrowska, M.H. Borawska, Concentration of selenium, zinc, copper, Cu/Zn ratio, total antioxidant status and c-reactive protein in the serum of patients with psoriasis treated by narrow-band ultraviolet B phototherapy: a case-control study, J. Trace Elem. Med. Biol. 44 (2017) 109–114.
[41] H. Yanagisawa, T. Kawashima, M. Miyazawa, T. Ohshiro, Validity of the copper/zinc ratio as a diagnostic marker for taste disorders associated with zinc deficiency, J. Trace Elem. Med. Biol. 36 (2016) 80–83.
[42] P. Ozturka, E.B. Kurutas, A. Ataseven, Copper/zinc and copper/selenium ratios, and oxidative stress as biochemical markers in recurrent aphthous stomatitis, J. Trace Elem. Med. Biol. 27 (2013) 312–316.