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Papillary Thyroid Carcinoma: A Malignant Tumor with Increased Antioxidant Defense Capacity

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Papillary thyroid carcinoma (PTC) is the commonest thyroid malignancy worldwide for which the radiation exposure is the most influential risk factor. The levels of oxidative stress in PTC are not well characterized on the tissue level. The objective of this study was to evaluate total oxidant status (TOS) and total antioxidant status (TAS) in PTC and benign goiter (BG) tissues and to examine their association with clinicopathological characteristics. Tumor and normal thyroid tissue samples were collected from 59 PTC patients, and goiter tissues were collected from 50 BG patients. TOS and TAS were quantified in the tissue homogenates by spectrophotometric assays. TOS values in tumor tissues did not differ significantly from normal and goiter tissues; however, PTC tissues have significantly higher TAS values than normal and goiter tissues. TOS values correlated with retrosternal growth in BG patients. The significant correlations were found between TOS and TAS values and thyroid function parameters. In 17 PTC patients with multiple tumor foci (multicentric phenotype), TAS values were significantly lower, compared to 42 patients with unicentric PTC. TAS and TOS are the most useful predictors of thyroid capsular invasion by PTC. The age, sex, body mass index, smoking, familial history of thyroid disease and nodule size did not influence TOS and TAS in PTC or BG patients. In conclusion, we show the profiles of TOS and TAS in PTC and BG tissues. Importantly, PTC tissues possess increased antioxidant capacity. The redox status influences the parameters of the thyroid function and tumor's biological behavior.

Keywords: benign goiter; oxidative stress index; papillary thyroid carcinoma; total antioxidant status; total oxidant status

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Introduction

Papillary thyroid carcinoma (PTC) is the most common follicular cell-derived malignant thyroid tumor, which comprising > 70% of all thyroid malignancies and its incidence has been increasing worldwide for the past few decades (Abd Elmageed et al. 2013). Environmental, genetic and hormonal etiological factors contribute to the development of PTC and radiation exposure has been recognized as the most influential risk factor (Hemminiki and Li 2003). The biologically indolent nature of PTC contributes to its excellent clinical prognosis, with > 90% survival rate at 20 years (Hwang et al. 2015). PTC patients most often present with a palpable thyroid nodule, while non-palpable nodules are incidentally revealed by ultrasonographic, CT or MRI examination. Small PTC nodules or microcarcinomas are < 1 cm in diameter and they are generally of no clinical relevance, particularly in younger patients who have a 20-year survival rate > 98% even with palpable tumors (Cooper et al. 2009). The most important aim in the clinical evaluation of patients with thyroid nodules is to discriminate thyroid cancer from benign nodules. Although this objective can be achieved with conventional diagnostic techniques including ultrasonography and fine needle aspiration biopsy (FNAB), in most patients, these diagnostic procedures cannot always provide reliable preoperative diagnosis (Nikita et al. 2016). Various studies have examined the molecular pathology of PTC while striving to provide reliable and informative diagnostic and prognostic biomarkers. However, only a limited number of studies have evaluated the role of oxidative stress in PTC carcinogenesis in situ. Oxidative reactions take place in all organs including the thyroid gland in which oxidative processes are fundamental for thyroid hormone synthesis. It is estimated that large amounts of reactive oxygen species (ROS) are produced in the thyroid under physiological con-

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ditions, justifying the statement that the thyroid gland is an organ of "oxidative nature" (Karbownik-Lewińska and Kokoszko-Bilska 2012). Physiological conditions are characterized by a balance between production and detoxification of free radicals, which involves antioxidative molecules and the process of compartmentalization of potentially toxic molecules (Kovacic and Edwards 2010). Various studies have examined the role of individual oxidant and antioxidant factors in the pathogenesis of benign and malignant thyroid diseases, however, their separate measurement is not only impractical but also holds little clinical importance (Wang et al. 2011). An overall oxidation state can be measured by assaying total oxidant status (TOS) and the combined effect of enzymatic and nonenzymatic antioxidants can be estimated by measuring total antioxidant status (TAS). The oxidative stress index (OSI) can be derived from these two parameters, and it provides overall insight into redox status (Erel 2004, 2005). This collective assessment of oxidant and antioxidant status is an improved way to evaluate the overall oxidation state than separate measurement of oxidants and antioxidants, which is time consuming and labor intensive.

The aim of this study was to examine the tissue levels of TOS, TAS and OSI for the first time in patients with PTC and those with benign goiter (BG). Evaluation of the dependence of these parameters upon various clinical and pathological characteristics was another important aspect of this research.

Materials and Methods

Patients and experimental design

Caucasian patients of any age and gender who underwent thyroid surgery during 2015 were divided in two cohorts: an experimental group of euthyroid patients with PTC (n = 59) and a control group of euthyroid patients with BG (n = 50) who were matched by age and gender with the PTC group. Postoperative samples of normal and tumor tissues were collected from each PTC patient and goiter tissue was collected from the BG patients The PTC tissue was excised from the center of the tumor and the normal tissue was excised from the thyroid gland at the greatest possible distance from the tumor. The normal tissue was defined as the self-control for each PTC patient, while the independent control group included the goiter tissues collected from the BG patients. Patients with chronic diseases such as neoplastic diseases, diabetes, unstable angina, unregulated hypertension or chronic infectious diseases were excluded from the study. The postoperative tissue samples were subjected to pathohistological examination by standard hematoxylin/eosin staining and the definitive diagnosis was obtained by two independent pathologists. TNM staging was performed according to the recommendations of the AJCC Cancer Staging Manual (Edge et al. 2010). Basic demographic and clinical data were also collected, including age, gender, BMI, smoking status, previous family history of thyroid disease, TNM stage, nodule size, capsular invasion, multicentricity, retrosternal propagation, plasma thyroid hormone levels (T3, T4, free T3 and free T4), plasma TSH and plasma thyroglobulin (TG) as well as titer of anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-TG) auto antibodies. Each patient gave written informed consent according to the 1964 Declaration of Helsinki. The Ethical Committee of the Clinical Center of Serbia approved the study (approval number 1575/7).

Chemicals and reagents

Chemicals used in the determination of TOS, TAS and protein concentration were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). Reagents for assaying thyroid hormone concentrations were purchased from DiaSorin S.p.A, (Saluggia, Italy), and ELISA kits were obtained from Omega Diagnostics (Alva, Scotland, UK). Disposable laboratory materials were obtained from Eppendorf (Hamburg, Germany).

Measurement of thyroid hormone concentrations and anti-thyroid antibodies

After overnight fasting, blood was drawn from the patients by antecubital venipuncture between 07:30 and 10:00 h. Approximately 5 ml of peripheral blood was collected into a BD Vacutainer SST II ADVANCE tube (Becton Dickinson, Rutherford, NJ, USA) for analysis of thyroid profiles. After 1 h, blood samples were centrifuged at 800 × *g* for 10 min, and serum samples were collected and stored at −20°C until analysis within 24 h. Thyroid hormone levels (T3, T4, free T3 and free T4) and TSH were assayed by a chemiluminescence assay method using a LIAISON analyzer with kits obtained from DiaSorin S.p.A. The concentrations of auto-antibodies were determined by ELISA, using the Apollo LB 913 microplate reader (Berthold Technologies GmbH & Co. KG, Bad Wildbad, Germany).

Tissue collection and preparation

The normal, tumor and goiter tissue samples were collected immediately after thyroidectomy. Approximately 5 mm^3 of tissues was excised from the thyroid gland, placed in sterile tubes and stored at −80°C until further manipulation. Each tissue specimen was thawed in 500 μl of 0.5M sterile phosphate buffer (pH7.5) and homogenized with a mechanical homogenizer (M-HOG-020, Labec, Marrickville, NSW, Australia) in sterile tubes, immersed in ice cold distilled water during the homogenization process. The homogenates were centrifuged at 8,161 × *g* for 10 min, and the supernatants were aliquoted and stored at −80°C for further biochemical analyses (Deja et al. 2013).

Measurement of the total protein

Total protein in the tissue homogenates was determined by the Bradford spectrophotometric method (Bradford 1976), using a Shimadzu, UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan). The results were expressed as the mean mg proteins per ml of tissue homogenate following three replications.

Measurement of TOS

TOS was determined according to the method of Erel (2005). The assay is based on the oxidation of ferrous to ferric ion in acidic medium in the presence of various oxidant species, and the detection of ferric ion by xylenol orange. Calibration and construction of a standard curve were performed using a hydrogen peroxide concentration gradient (0-100 μM). The absorbance at 560 nm was measured on a spectrophotometer. The results were expressed as the mean micro molar equivalent of hydrogen peroxide per mg of tissue proteins (μmol H_2O_2 Eq./mg proteins) following three replications.

Measurement of TAS

TAS was determined according to the method of Erel (2004). The method is based on decoloration of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation [ABTS(*+)] by various antioxidants. Calibration and construction of standard curve were performed using a Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) concentration gradient (0-100 μM). The absorbance at 660 nm was measured spectrophotometrically. The results were expressed as the mean micromolar equivalents of Trolox per mg of tissue proteins (μmol Trolox Eq./mg proteins) following three replications.

Calculation of OSI

OSI was defined as the ratio of TOS to TAS, and it was displayed by arbitrary unit = TOS (μmol H_2O_2 Eq./mg proteins)/TAS (μmol Trolox Eq./mg proteins) (Erel 2004).

Statistical analysis

All statistical analyses were performed in IBM SPSS Statistics for Windows v20 (IBM Corp., Armonk, NY, USA). The resulting data are presented as the Mean ± Standard Error (SE). The data normality distribution was examined using the Kolmogorov-Smirnov test. For assessment of significant difference between experimental groups, the Chi-squared, Student's t, Mann-Whitney and Wilcoxon signed rank tests were used. The Pearson and Spearman tests were used for correlation analysis of biochemical parameters with clinical and demographic characteristics of patients. Simple and Multiple linear regression tests were used for estimating the predictive value of single and multiple parameters for different clinical characteristics. The Kruskal-Wallis H test was used to examine the difference in values of biochemical parameters between different TNM stages of PTC patients. Two-step cluster analysis with log-likelihood distance measurement was used to determine the TOS, TAS and OSI data clustering pattern based on the patient characteristics and clinical parameters according to the Schwarz's-Bayesian criterion. Differences were

Table 1. The clinical features and laboratory findings of the PTC and the control group patients.

Parameter	PTC group	BG group	P-value
Number of patients	n = 59	n = 50	
Age (years)	54 ± 14.1[a]	63 ± 15[b]	0.18[‡]
Gender (male/female)	18/41	12/38	0.07[†]
BMI (kg/m^2)	22.31 ± 3.7[a]	21.84 ± 3.1[a]	0.42[§]
Smoking (yes/no)	15/44	16/34	0.08[†]
Heredity (yes/no)	16/43	17/33	0.12[†]
Nodule size (cm)	3.19 ± 0.19[a]	3.57 ± 0.22[a]	0.54[§]
Capsular invasion (yes/no)	18/41	/	/
Multicentricity (yes/no)	17/42	/	/
Retrosternal (yes/no)	7/52	9/41	0.07[†]
T3 (nmol/l)	1.72 ± 0.46[b]	1.81 ± 0.09[a]	0.11[‡]
T4 (nmol/l)	108.45 ± 1.89[a]	115.71 ± 4.46[a]	0.24[§]
TSH (μIU/ml)	1.18 ± 1.73[b]	1.18 ± 0.16[a]	0.71[‡]
FT3 (pg/ml)	4.8 ± 0.16[a]	4.76 ± 0.27[a]	0.90[§]
FT4 (pg/ml)	14.8 ± 0.46[a]	15.02 ± 0.63[a]	0.78[§]
Thyroglobulin (ng/ml)	160.98 ± 252.50[b]	182.94 ± 45.99[a]	0.94[‡]
anti-TPO-ab (IU/ml)	15.4 ± 11.2[b]	10.04 ± 21.88[b]	0.09[‡]
anti-TG-ab (IU/ml)	20.0 ± 25.86[b]	20.0 ± 8.06[b]	0.51[‡]

[a]For normally distributed data the results are shown as the arithmetic mean ± SE.
[b]Data with a skewed distribution are summarized as median with interquartile range.
[†]Chi squared test.
[‡]Mann-Whitney test.
[§]Independent samples t-test.

considered to be statistically significant at $P < 0.05$.

Results

Patient characteristics

The basic demographic, social, and clinical characteristics of patients in both groups are presented in Table 1. The experimental and control groups were matched by age and gender, and there were no differences in their smoking status. The clinical data showed that there were also no significant differences between the groups that could be confounding factors affecting the examined biochemical parameters.

The TNM stages of the PTC patients group are shown in Table 2. Most of PTC patients had T1 tumor stage, the lymph node metastases were detected in only one fifth of them, and distant metastatic deposits were not detected in any patient.

Table 2. TNM stage of the PTC patients group.

TNM	Stage	Number of patients
T	1	29 (49.38 %)
	2	14 (23.85 %)
	3	15 (25.75 %)
	4	1 (0.02 %)
N	0	12 (20.33 %)
	1	7 (11.86 %)
	x	40 (67.81 %)
M	0	59 (100 %)
	1	0

Total Oxidant Status

The TOS values in the PTC and control groups are presented in Fig. 1. TOS did not differ significantly among the three groups, but the levels in tumor tissues tended to be between those in normal and BG tissues.

The plasma T4 concentrations directly correlated with TOS in tumor tissues of PTC patients ($\rho = 0.398$, $P < 0.01$; Fig. 2A). There was a noticeably positive correlation ($\rho = 0.508$, $P < 0.005$) between plasma thyroglobulin concentrations and TOS in normal tissue of PTC patients (Fig. 2B). The TOS values in BG positively correlate ($\rho = 0.314$, $P < 0.05$) with the existence of goiter retrosternal propagation.

Total Antioxidant Status

The plasma T3 concentrations negatively correlated ($\rho = -0.477$, $P < 0.01$) with TAS values in the BG tissues (Fig. 2C), whereas the plasma TSH concentrations positively correlated with TAS values ($\rho = 0.334$, $P < 0.05$) in the same group of patients (Fig. 2D). There was a negative correlation ($\rho = -0.331$, $P < 0.05$) between the nodule size of BG tissues and TAS values (Fig. 2E).

The TAS values in the PTC and control groups are presented in Fig. 3. The highest TAS values were observed in PTC tissue, and they differed significantly from those in normal and goiter tissues. The antioxidative capacity of the normal surrounding tissues in PTC patients was significantly lower than the goiter tissues. In addition, the TAS values were significantly lower ($P < 0.01$) in tumor tissues of patients with multicentric PTC than in patients with uni-

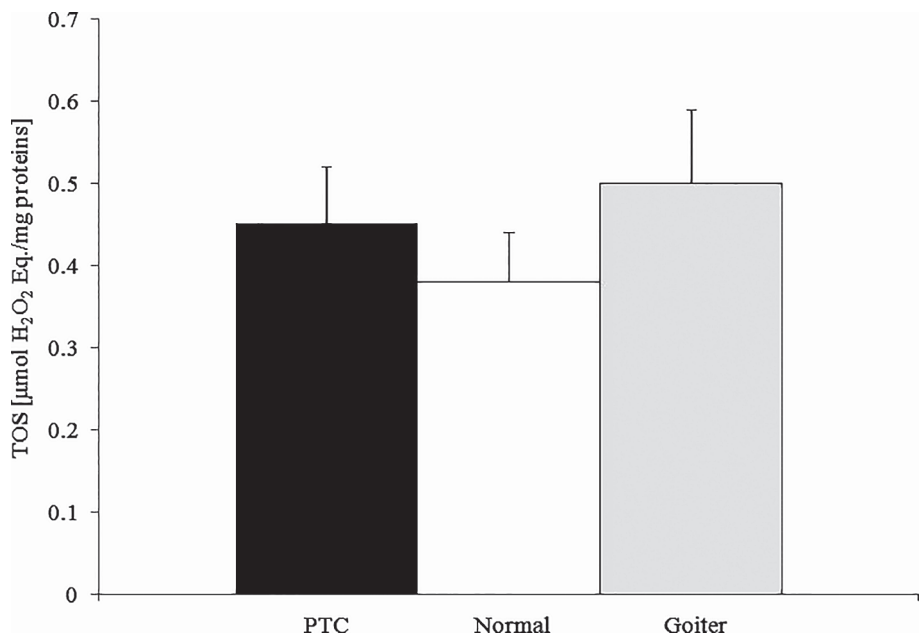

Fig. 1. TOS in PTC and normal surrounding tissues of 59 PTC patients and in BG tissue of 50 BG patients. There were no statistically significant differences ($P > 0.05$) in TOS levels between these three groups.

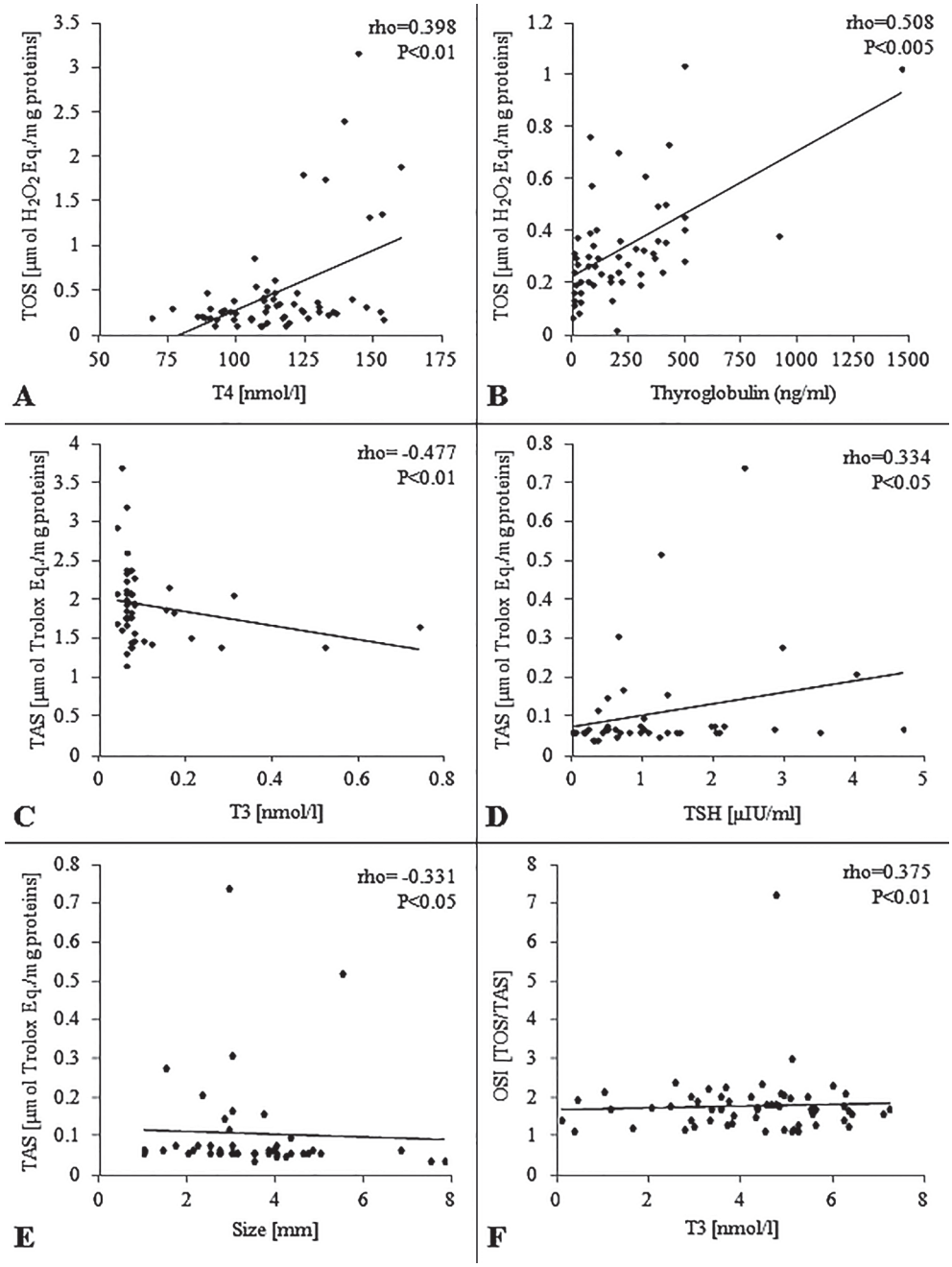

Fig. 2. Statistically significant correlations found between TOS, TAS and OSI and clinical parameters. A. Positive correlation between plasma T4 concentrations and TOS values in tumor tissue of 59 PTC patients. B. Plasma thyroglobulin concentrations positively correlate with TOS values in normal tissues of 55 PTC patients (plasma thyroglobulin concentration values were missing for four patients). C. Plasma T3 concentrations negatively correlate with TAS values in goiter tissues of 50 BG patients. D. Plasma TSH concentrations positively correlate with TAS values in goiter tissues of 50 BG patients. E. Negative correlation between nodule size of BG and TAS values of 50 BG patients. F. Positive correlation between plasma T3 concentrations and OSI in tumor tissues of 59 PTC patients.

centric PTC (Fig. 4). Additionally, there was a positive correlation ($\rho = 0.324$, $P < 0.01$) between TAS values in tumor tissues and multicentric dissemination.

Oxidative Stress Index

The plasma T3 concentrations positively correlated ($\rho = 0.375$, $P < 0.01$) with OSI values in tumor tissues of PTC patients (Fig. 2F). The OSI values in the PTC and BG groups are presented in Fig. 5. The lowest OSI was observed in the PTC tumor tissue, and it significantly differed from the normal surrounding tissue. The OSI in BG was highest, but not significantly different from the other groups, due to high interindividual variability.

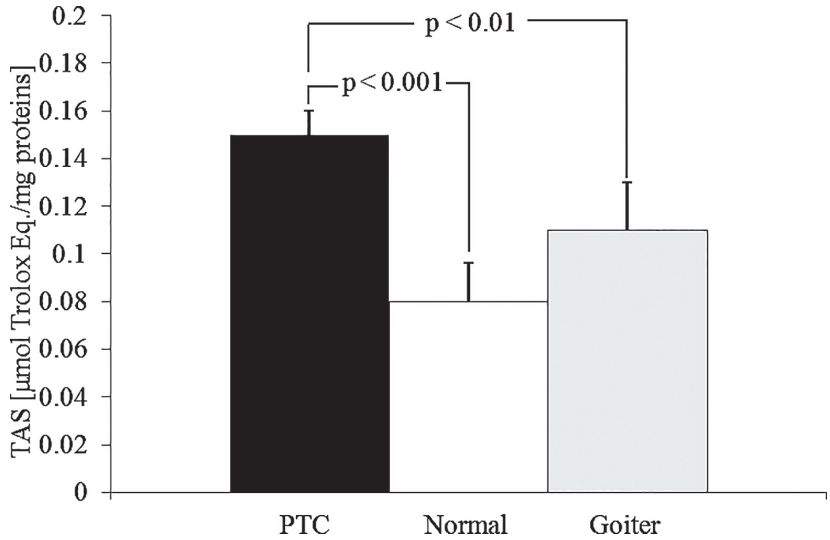

Fig. 3. TAS s in PTC tumor and normal surrounding tissues of 59 PTC patients and BG tissues of 50 BG patients. The TAS levels were highest in the tumor tissues and they differed significantly from the normal surrounding tissue ($P < 0.001$) and goiter tissue ($P < 0.01$). Normal and BG tissues do not differ significantly.

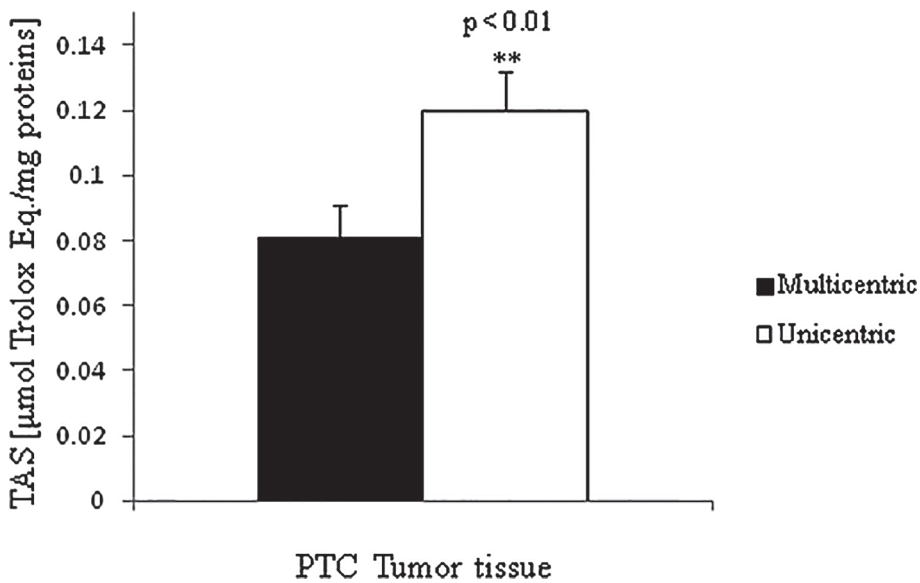

Fig. 4. TAS in tumor tissues of multicentric (n = 17) and unicentric (n = 42) PTC. The levels were significantly lower ($P < 0.01$) in tumors with multicentric intrathyroid dissemination.

Relations between biochemical, demographic and clinical parameters

TOS, TAS and OSI values in PTC patients with different TNM stages did not differ significantly according to Kruskal-Wallis H test.

There were no significant differences in the levels of redox parameters between subgroups created according to gender, smoking status, heredity, retrosternal propagation, multicentricity or capsular invasion.

No correlations were found between age, sex, BMI, smoking status, heredity or TNM stage and any biochemical parameter included in the study.

Association between TOS, TAS and OSI and parameters of thyroid function

The relationship between TOS, TAS and OSI altogether and clinical parameters which reflect thyroid function was investigated. By using a multiple linear regression model the predictive value of TOS, TAS, and OSI obtained in examined tissues on different clinical parameters were examined.

Association between plasma thyroglobulin levels and TOS, TAS and OSI

Significant association was found in the PTC patient

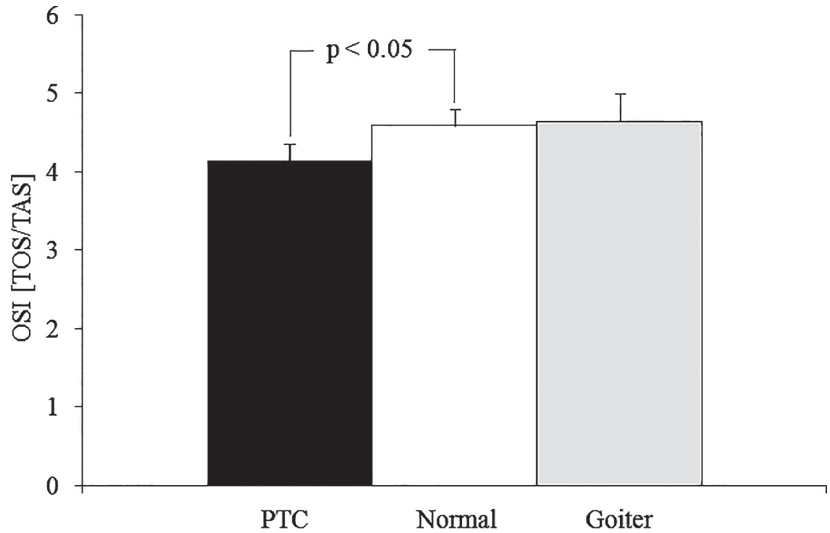

Fig. 5. OSI in tumor and normal surrounding tissues of 59 PTC patients and in BG tissues of 50 BG patients. The tumor tissues had the lowest OSI which differed significantly ($P < 0.05$) from the OSI of normal tissues. The OSI of BG tissues did not differ significantly from the PTC tissues due to high interindividual variability.

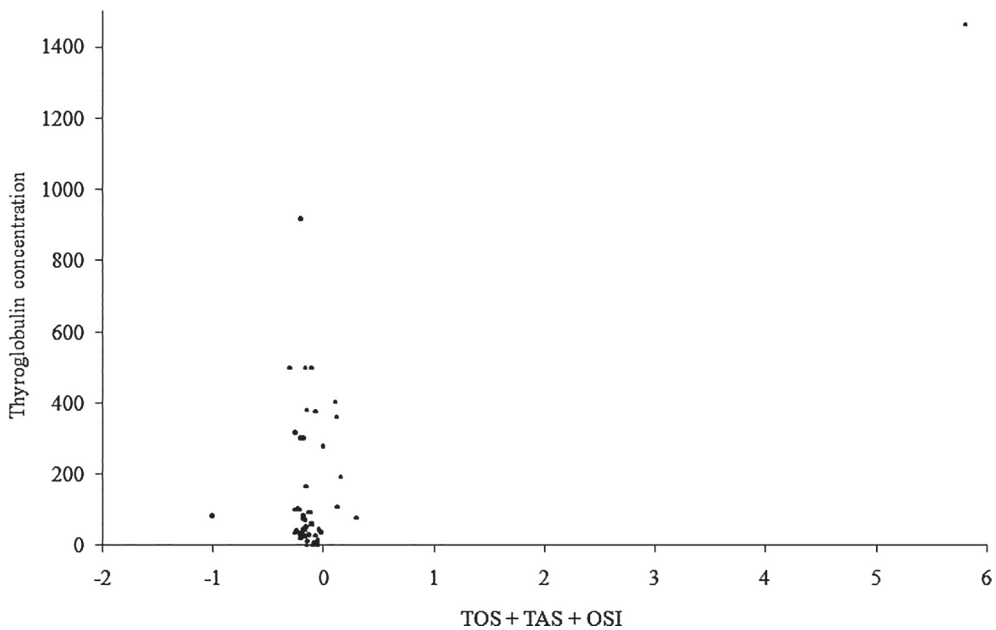

Fig. 6. The multiple linear regression plot showing the dependence between plasma thyroglobulin concentration and tumor levels of TOS, TAS and OSI in 55 PTC patients. "TOS + TAS + OSI" represents three explanatory variables which were used together for predicting the values of plasma thyroglobulin concentrations. Circulating thyroglobulin levels and tumor levels of TOS, TAS and OSI showed direct dependence in PTC patient group ($P < 0.001$, $R^2 = 0.497$, S.E. = 218.41). The findings indicate that all three biochemical parameters can be collectively used for prediction of plasma thyroglobulin concentrations by multiple linear regression model. Plasma thyroglobulin concentration values were missing for four patients.

group where it was shown that plasma thyroglobulin concentration and tumor levels of TOS, TAS and OSI taken together are dependent ($P < 0.001$, $R^2 = 0.497$, SE = 218.41), which was modeled by the regression equation: thyroglobulin = (−1912.12) TOS + (9002.11) TAS + (105.93) OSI – 328.92. The regression plot is shown in Fig. 6. The data were missing for four PTC patients. Further analysis using a simple linear regression versus multiple linear regression models showed that the combination of TOS, TAS and OSI has better predictive results than their individual influence on plasma thyroglobulin concentration, based on the higher R^2 and lower SE values inferred by the multiple linear regression.

Association between plasma T3 levels and TOS, TAS and OSI

In addition, there was a statistically significant depen-

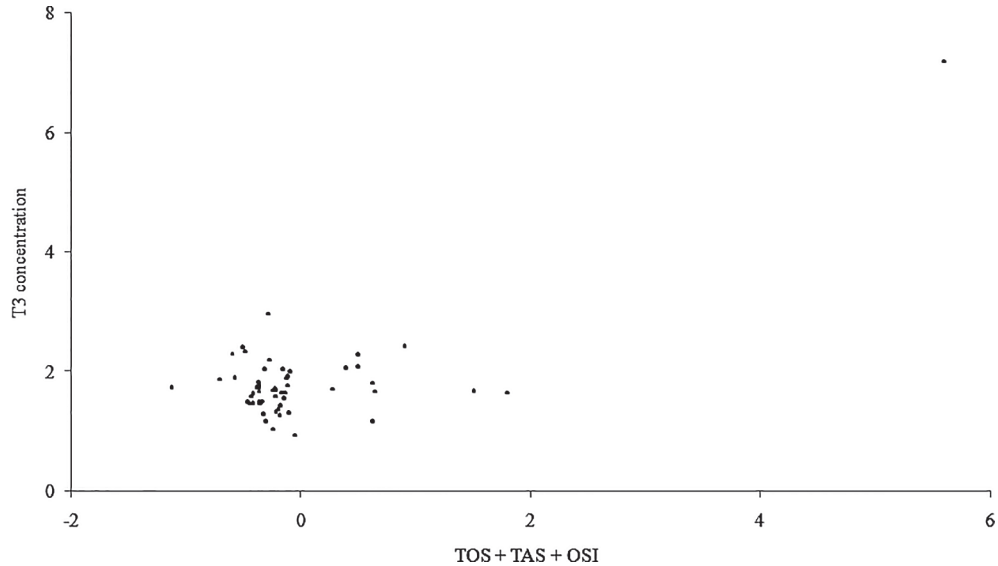

Fig. 7. The multiple linear regression plot showing the dependence between plasma T3 concentration and tumor levels of TOS, TAS and OSI in 59 PTC patients.
"TOS + TAS + OSI" represents three explanatory variables which were used together for predicting the value of plasma T3 concentration. Circulating T3 levels are directly dependent upon tumor levels of TOS, TAS and OSI in PTC patient group ($P < 0.001$, $R^2 = 0.602$, SE = 0.571). The findings indicate that all three biochemical parameters can be used collectively for prediction of plasma T3 concentration by multiple linear regression model.

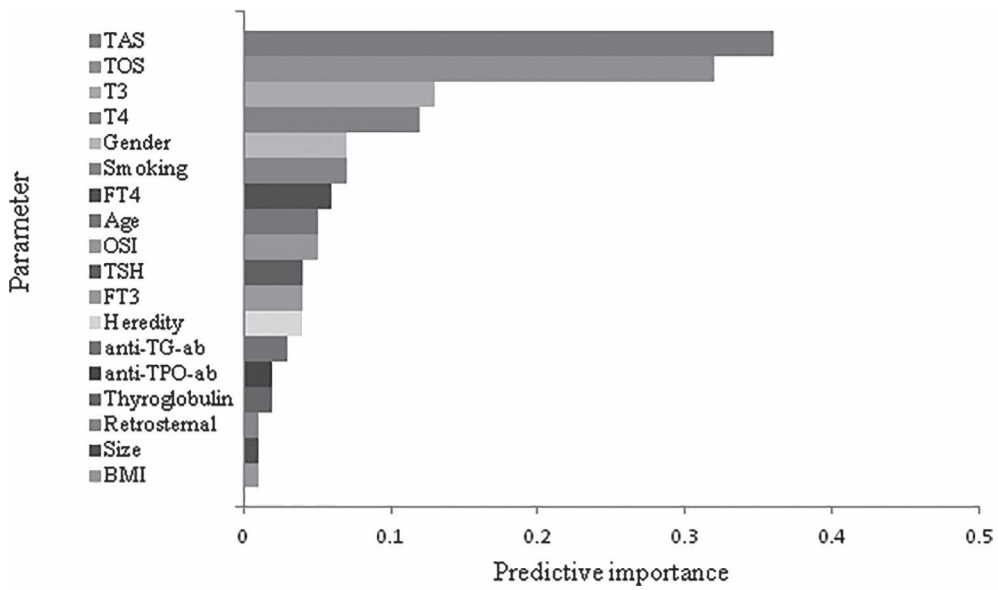

Fig. 8. Predictive importance values of biochemical and clinical parameters in two clusters of PTC patients with and without tumor capsule invasion.
The TAS and TOS had the greatest predictive value of the examined parameters in PTC patients with the tumor capsular invasion. The data were obtained by log-likelihood clustering based on the Schwarz-Bayesian criterion of the PTC patient group: patients with tumor capsular invasion (n = 18) and without tumor capsule invasion (n = 41).

dence in the PTC patient group between plasma T3 concentration and tumor levels of TOS, TAS and OSI taken together ($P < 0.001$, $R^2 = 0.602$, SE = 0.571) and it was modeled by the regression equation: T3 = (−11.05) TOS + (65.32) TAS + (0.664) OSI – 2.194. The regression plot is shown in Fig. 7. The simple linear regression versus multiple linear regression models showed that the combination of TOS, TAS and OSI have better predictive results than their individual influence on plasma T3 concentration, based on the higher R^2 and lower SE values inferred by the multiple linear regression.

Predictive significance of TOS, TAS and OSI on patients' clinical characteristics

Cluster analysis was performed to infer the clustering of TOS, TAS and OSI values around the different patient characteristics and clinical features of PTC and BG. The good clustering model (cluster quality score 0.62) was achieved for the existence or absence of tumor capsule invasion (cluster size ratio = 1.68). TAS and TOS values were found to have the highest direct predictive value for invaded tumor capsule in patients with PTC (Fig. 8).

Discussion

Considerable ROS production is necessary for the physiological functions of the thyroid gland, particularly the synthesis of thyroid hormones. The main source of ROS in all cells appears to be mitochondrial respiration, though recent data reveal that NADPH oxidases (NOX) allow controlled ROS generation at the subcellular level (Fortunato et al. 2014). Several decades ago, high concentrations of hydrogen peroxide were detected on the thyrocyte apical surface, where thyroid hormone biosynthesis takes place, and later the enzymatic source of hydrogen peroxide involved in thyroid hormone biosynthesis was characterized by cloning of genes encoding NADPH oxidases: dual oxidases 1 and 2 (DUOX1 and DUOX2) (Carvalho and Dupuy 2013). Besides its physiological role, oxidative stress appears to accompany thyroid carcinogenesis and to influence disease progression (Young et al. 2010; Stanley et al. 2016; Ece et al. 2013; Yanagawa et al. 1999). Damaging effect in thyroid tissue occur when there is the regulation misbalance between the produced free radicals and antioxidants, leading to apoptosis via activation of apoptosis signal-regulating kinase 1 (ASK1) (Du et al. 2010). Papillary carcinogenesis is associated with increased serum levels of oxidants induced by the DNA damage adduct 8-hydroxy-2′-deoxyguanosine (8-OHdG), which remains high after surgery (Tabur et al. 2015). Oxidative stress can be experimentally characterized by assaying numerous enzymatic and non-enzymatic compounds that contribute to the cell's redox status. The general extent of oxidative stress can be assessed by determining the TOS, TAS and OSI in a variety of biological materials. The literature is rich in studies examining TOS, TAS and OSI in a wide spectrum of human diseases, but, to our knowledge, this is the first report of their levels in PTC and BG tissue. TOS levels in the PTC tissue were not significantly increased compared to normal and BG tissue, indicating that the moderate oxidant production by PTC cells which does not differ from the benign tumor is in the concordance with favorable clinical outcome of PTC. In fact, BG tissue produced more oxidants than normal and tumor tissue of PTC patients. Erdamar et al. (2010) observed increased pro-oxidant activity such as lipid peroxidation and impaired antioxidant defense mechanisms in both tissue of patients with non-toxic and toxic multinodular goiter and those with PTC. In contrast, TAS values were significantly higher in PTC tumor tissue than in nearby normal tissue and BG tissue. This interesting fact could explain the slow carcinogenesis process which is often driven by augmented oxidative stress via specific redox signaling. The PTC tissues appear to have greater capability to scavenge free radicals than normal and goiter tissues. The biological feature of PTC seems to be unique among human malignancies. The OSI was significantly higher in normal tissue of PTC patients, probably due to the peritumoral lymphocyte infiltration found in this type of thyroid carcinoma (Villagelin et al. 2011). The benign nature of colloid goiter is consistent with the lower OSI values in tumors than in normal tissue. The revealed correlation between parameters suggests that circulating levels of thyroid hormones affect the redox status in all groups. In the PTC group, T4 appears to stimulate pro-oxidant production in tumor tissue, whereas in the BG group, T3 appears to down-regulate antioxidant defense capacity. In normal tissue of PTC patients, the increased oxidant environment directly correlates with the thyroglobulin levels in circulation. The extent of thyroid metabolic function is reflected in the levels of circulating T3 and thyroglobulin, upon which the intrathyroid redox status is dependent. Romitti et al. (2016) demonstrated that the increase in circulating T3 is caused by higher deiodinase activity in PTC. Larger goiters that have a tendency to grow and to acquire retrosternal position are characterized by increased oxidants production, whereas the negative correlation between TAS and goiter size reveals the importance of appropriate antioxidant capacity in the prevention of goiter growth. The importance of antioxidant defense has been previously demonstrated as impaired antioxidant defense that leads to the increased growth, DNA damage and malignant transformation of benign goiters (Paschke 2011). Intraglandular PTC dissemination appears to be influenced by TAS, which was significantly lower in tumor tissue of patients with multicentric PTC. The redox system is highly sensitive to cellular changes, such as ageing and transformation, and for that reason can function as a sensor in cell differentiation and carcinogenesis (Cammarota et al. 2015). The reduced levels of TAS in multicentric PTC may be associated with the down regulation of the thiol-specific antioxidant proteins peroxiredoxins (PRDXs), which are decreased in PTC and are highly correlated with tumor dedifferentiation and progression (Nicolussi et al. 2014). Another novel finding was that the capsular invading tumor phenotype appears to be most directly dependent on TAS and TOS of the all other important clinical and pathological parameters examined in this study.

Our results suggest that PTC tissues have sufficient antioxidative capacity to counter the detrimental effects of thyroid-produced free radicals. The enhanced antioxidative defense capacity can mask the increased production of free radicals in PTC tissues, and this could be a possible explanation for the lack of significant differences in TOS between tumor and control tissues. The well developed vascularization of the thyroid clears the thyroid tissue of free radicals

and deposits them into circulation, which is the probable reason why previous studies found increased serum oxidant concentrations. In addition, the potential influence of confounding factors for oxidative stress, such as smoking status and co-morbidities, on TOS and TAS values were not evaluated in previous studies (Wang et al. 2011; Tabur et al. 2015). Based on the present results, we propose that future studies should carry out further characterization of oxidant/antioxidant status in PTC and BG tissues by assaying the specific enzymatic and non-enzymatic components of the redox system. Although PTC is easily distinguishable and confined from the rest of the thyroid tissues and the normal tissues of PTC patients were collected as far from the tumor as possible, we cannot be absolutely sure that the biochemical events in normal tissues of PTC patients are entirely equivalent to these events in the thyroid gland of healthy individuals.

In conclusion, this study is the first to reveal the profiles of TOS, TAS and OSI in PTC and BG tissues. The PTC tumor tissue does not show the existence of oxidative stress compared with nearby normal tissue and BG. The increased TOS in BG is associated with its retrosternal growth. The tumor tissue showed increased antioxidative capacity, whereas it decreased in multicentric PTC localization. Additionally, the capsular invasion phenotype was highly influenced by TAS and TOS. The redox status is greatly intertwined with thyroid hormone production and it is influenced by the circulating amounts of T3, T4 and thyroglobulin. The age, sex, BMI, smoking status, familiar history of thyroid disease and the nodule size do not appear to influence TOS and TAS in normal or pathologically altered thyroid tissues.

Conflict of Interest

The authors declare no conflict of interest.

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