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Copper as Ancillary Diagnostic Tool in Preoperative Evaluation of Possible Papillary Thyroid Carcinoma in Patients with Benign Thyroid Disease

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Abstract Preoperative diagnosis of papillary thyroid carcinoma (PTC) comprises numerous diagnostic procedures which are mostly applicable in tertiary institutions. Normal thyroid function depends on the presence of many trace elements and copper (Cu) and zinc (Zn) are some of those. The study is based on retrospective review of 118 patients with preoperatively diagnosed benign thyroid disease (BTD) and 12 with PTC, who underwent thyroid surgery at the Center for Endocrine Surgery Clinical Center of Serbia, Belgrade, between 2010 and 2012. The objective was to evaluate concentrations of Cu and Zn in serum as possible prediction markers for PTC in patients who underwent surgery for preoperatively diagnosed BTD. Concentrations of Cu and Zn ions in serum were measured using atomic absorption spectrophotometer. Data were analyzed using methods of descriptive statistics, Anova and *t*-test ($p<0.05$ was considered statistically significant). Definitive pathohistological findings revealed PTC in 23 (19.5 %) and papillary microcarcinoma—mPTC in 13 (11.0 %) of BTD patients. The concentrations of Cu ions in serum of PTC patients as well as in serum of patients with mPTC were significantly higher than in serum of BTD patients ($p<0.05$). The concentrations of Zn ions and Cu/Zn ratio in serum of PTC and mPTC patients were not significantly higher than in serum of BTD patients. The concentration of Cu ions in serum of patients before thyroid surgery can be useful, easy available, and a low-cost tool in prediction of preoperatively undiagnosed PTC in patients with BTD.

Keywords Papillary thyroid carcinoma · Benign thyroid disease · Copper · Serum

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Introduction

Papillary thyroid carcinoma (PTC) is the most common thyroid carcinoma [1, 2]. Preoperative diagnosis of PTC encompasses various diagnostic procedures which are often not available in all medical institutions.

Normal thyroid function depends on the presence of many trace elements for both synthesis and metabolism of thyroid hormones [3, 4]. Copper (Cu) and zinc (Zn) are some of those elements. Cu has many important physiological functions as redox active element in maintaining thyroid activity and lipid metabolism. It is essential for preserving the strength of the skin, blood vessels, epithelial, and connective tissues. Cu is incorporated in the production of hemoglobin, myelin, and melanin and is essential for thyroid gland functioning [5], by stimulating production of thyroxin hormone (T4), and preventing overabsorption of T4 by controlling the calcium levels. Cu can act both as antioxidant and pro-oxidant. As an antioxidant, Cu scavenges or neutralizes free radicals and may reduce or prevent some of the damage caused by them [6, 7]. A high concentration of Cu can induce growth proliferation and cancer by damaging DNA (deoxyribonucleic acid) with toxic free hydroxyl radicals [8].

Zinc (Zn) function can be described as follows: catalytic, regulatory, and structural. Zn homeostasis is controlled by the coordinated actions of Zn transporters which regulate intracellular and extracellular Zn concentration and distribution. Zn is important for normal thyroid homeostasis. Its function is complex and may include effects on both synthesis and mode of hormones action [9, 10]. Thyroid hormone binding transcription factors, which are essential for modulation of gene expression, contain Zn bound to cysteine residues. In cultured cells, very strong chelators of Zn are required to influence binding of transcription factors to DNA. In the thyroid gland, transcription factor 2, which interacts with the promoters for thyroglobulin and thyroperoxidase genes, is a Zn-containing protein [11]. Low Zn concentration is associated with decreased thyroid hormone level, and this association requires further investigations.

Cu and Zn ions are cofactors of superoxide dismutase 1, which prevents the onset and progression of tumors through mechanisms of cell protection against free radicals production [12].

The objective of our study was to evaluate the significance of Cu and Zn ions concentrations and Cu/Zn ratio in serum of PTC and benign thyroid disease (BTD) patients.

Material and Methods

Subject Selection and Clinical Data

Following approval by the Ethics Committee of the Center for Endocrine Surgery, Clinical Center of Serbia, 118 consecutive patients who had total thyroidectomy for benign thyroid disease (BTD), and 12 for PTC were selected from Center for Endocrine Surgery database in the 3-year period (2010–2012).

On the definite pathohistological finding, PTC was diagnosed in 23 (19.5 %) and papillary microcarcinoma—mPTC in 13 (11.0 %) of BTD patients. Consequently, patients were divided into four groups: 35 PTC, 13 mPTC, and 82 BTD patients and 8 healthy thyroids as control group.

Tumors were staged according to the American Joint Committee on Cancer (AJCC) sixth edition TNM staging system [13].

Instrumentation and Chemicals

A PerkinElmer AAS spectrophotometer 2380 (air/acetylene flame, appropriate hollow cathode lamp for absorption technique, deuterium lamp for back ground correction at the recommended current and conditions) was employed to measure the metal content of serum samples. In order to minimize the interference effects, calibration solutions were prepared as multielement standards (Cu and Zn), by dilution and mix of appropriate volumes of stock standard solutions (Merck, p.a. 1 g/L for each analyzed metal).

The concentrations of elements in these multiple element calibration solutions were analogous to AAS linear range determination for each of analyzed metals. The density of the serum samples is different compared with density standard solutions for calibration. Therefore, the mixed standard solutions in 5 and 10 % glycerol (*v*/*v*) were prepared.

All chemicals were Merck's, p.a. grade, unless it was stated otherwise. Deionized water with specific conductivity of 0.05 μS/cm was applied for washing laboratory vessels and solution preparation.

Quality Control

Triplicate serum samples were provided as a part of the quality control procedure. In the aim to annul influences caused by reagents, for all measurements, blanks were prepared and analyzed in the very same way as the samples (reagents without samples). For determinations of metals' concentrations in samples, calibration curve method was applied. The analytical precision, expressed as relative standard deviation, was routinely between 3 and 7 %. The analytical detection limits (triple standard deviation of the baseline noise/sensitivity) for each element were 0.025 ppm for Cu and 0.008 ppm for Zn.

Statistical Analysis

Cu and Zn sera concentrations are expressed as mean±SD. Differences in mean values of continuous variables between groups were tested using ANOVA, *t*-test, and Tukey's test ($p<0.05$ was considered statistically significant).

Results

Concentrations of Cu and Zn Ions and Cu/Zn Ratio

In PTC patients the median value of Cu and Zn ions concentration was 0.970±0.010 and 0.620±0.006 mg/L, respectively and 1.74 for Cu/Zn ratio. Majority of patients (65.7 %) was in stage II (Table 1).

In mPTC patients, the median value of Cu and Zn ions concentration was 0.920±0.009 and 0.570±0.006 mg/L, respectively and 1.78 for Cu/Zn ratio (Table 2).

In BTD patients, the median value of Cu and Zn ions concentration was 0.820±0.008 and 0.600±0.006 mg/L, respectively and 1.51 for Cu/Zn ratio.

The concentrations of Cu ions in serum of PTC patients as well as of patients with mPTC were significantly higher than in serum of BTD patients ($p<0.05$) (Fig. 1).

Table 1 The median value of Cu and Zn ions concentration, the Cu/Zn ratio, and tumor stage of PTC patients

Sample PTC	Stage	Cu (mg/L)	Zn (mg/L)	Cu/Zn
1	II	0.850±0.008	0.780±0.009	1.09
2	II	1.040±0.012	0.680±0.008	1.53
3	II	0.812±0.009	0.607±0.006	1.34
4	I	0.990±0.010	0.700±0.007	1.41
5	II	1.110±0.010	0.500±0.004	2.22
6	II	0.970±0.010	0.610±0.006	1.59
7	II	0.880±0.009	0.770±0.008	1.14
8	II	0.855±0.008	0.518±0.005	1.65
9	II	1.150±0.011	0.660±0.007	1.74
10	II	1.360±0.014	0.720±0.007	1.89
11	II	0.983±0.010	0.318±0.003	3.09
12	III	1.026±0.010	0.619±0.006	1.66
13	I	0.855±0.008	0.708±0.007	1.21
14	II	0.940±0.009	0.507±0.005	1.85
15	I	0.940±0.009	0.485±0.005	1.94
16	II	1.368±0.014	0.864±0.009	1.58
17	II	0.541±0.005	0.385±0.004	1.41
18	II	0.855±0.008	0.663±0.007	1.29
19	II	0.983±0.010	0.407±0.004	2.35
20	II	1.111±0.011	0.652±0.006	1.70
21	II	1.026±0.010	0.719±0.007	1.43
22	I	0.780±0.008	0.520±0.005	1.50
23	III	1.060±0.010	0.770±0.008	1.38
24	II	0.855±0.009	0.730±0.007	1.17
25	II	1.070±0.010	0.920±0.009	1.16
26	II	0.920±0.009	0.690±0.007	1.33
27	I	0.910±0.009	0.850±0.008	1.07
28	II	0.855±0.009	0.251±0.002	3.41
29	I	0.940±0.009	0.563±0.006	1.67
30	I	0.684±0.007	0.385±0.004	1.78
31	II	0.897±0.009	0.541±0.005	1.66
32	III	1.667±0.017	0.262±0.003	6.36
33	III	1.020±0.010	0.855±0.009	1.19
34	II	0.855±0.009	0.730±0.007	1.17
35	III	0.641±0.006	0.596±0.006	1.08
Median value	–	*0.970±0.010*	*0.620±0.006*	*1.74*

The concentrations of Zn ions in serum of PTC and mPTC patients were not significantly higher than in serum of BTD patients.

Correlation of the different tumor stages and the control group with concentrations of Cu and Zn ions and Cu/Zn ratio has shown in Fig. 2. The concentration of Cu ions was significantly higher $p<0.001$ in stages 2 and 3, but $p<0.01$ in stage 1. Cu/Zn ratio was statistically significant $p<0.05$ in stage 2.

Concentration of Cu ions in mPTC patients was significantly higher $p<0.001$ than in the control group.

Table 2 The median value of Cu and Zn ions concentration and the Cu/Zn ratio of mPTC patients

Sample mPTC	Cu (mg/L)	Zn (mg/L)	Cu/Zn
1	0.940±0.009	0.396±0.004	2.37
2	1.154±0.011	0.396±0.004	2.91
3	1.040±0.010	0.750±0.007	1.39
4	0.741±0.007	0.600±0.006	1.24
5	0.900±0.009	0.700±0.007	1.29
6	0.983±0.10	0.463±0.005	2.12
7	0.855±0.009	0.295±0.003	2.90
8	0.830±0.008	0.726±0.007	1.14
9	0.983±0.010	0.697±0.007	1.41
10	0.730±0.007	0.684±0.007	1.07
11	1.068±0.011	0.429±0.004	2.49
12	0.983±0.010	0.697±0.007	1.41
13	0.769±0.008	0.541±0.005	1.42
Median value	*0.920±0.009*	*0.570±0.006*	*1.78*

Discussion

Copper is believed to be the switch that turns on the angiogenesis process in tumor cells. Abnormally high serum Cu levels are found in the patients with many types of progressive tumors, making Cu an obligatory cofactor in angiogenesis process [14]. Some authors found that in breast and lung cancer, the concentrations of Cu and Zn ions were higher in malignant than in normal tissue [15–22].

Sosa et al. [23] demonstrated a significant inverse relationship between the volume of thyroidectomise performed by individual surgeons and complication rates, postoperative length of stay, and hospital charges.

There are several publications stating that the Cu/Zn ratio in the blood of patients with thyroid cancer was significantly higher than in other thyroid diseases. The concentration of Zn ions in the malignant thyroid tissue was significantly lower than in benign thyroid tissue, implying involvement of these ions in the carcinogenic process [24].

In a review by Blazevicz et al. [25], the concentrations of some trace elements such as Cu, Zn, manganese (Mn), and iron (Fe) were significantly higher in the control group (healthy thyroid) in comparison with the group of BTD.

Przybylik-Mazurek et al. [26] reported higher Cu concentrations in PTC patients compared with controls and patients with Hashimoto disease. They also found that in PTC patients, the parameter of the Zn/Cu ratio demonstrated reciprocally arranged statistically significant difference.

Mittag et al. [27] demonstrated serum Cu concentration as a novel biomarker for resistance to thyroid hormone and showed that serum Cu levels were regulated by thyroid hormone, which stimulated synthesis and export of hepatic

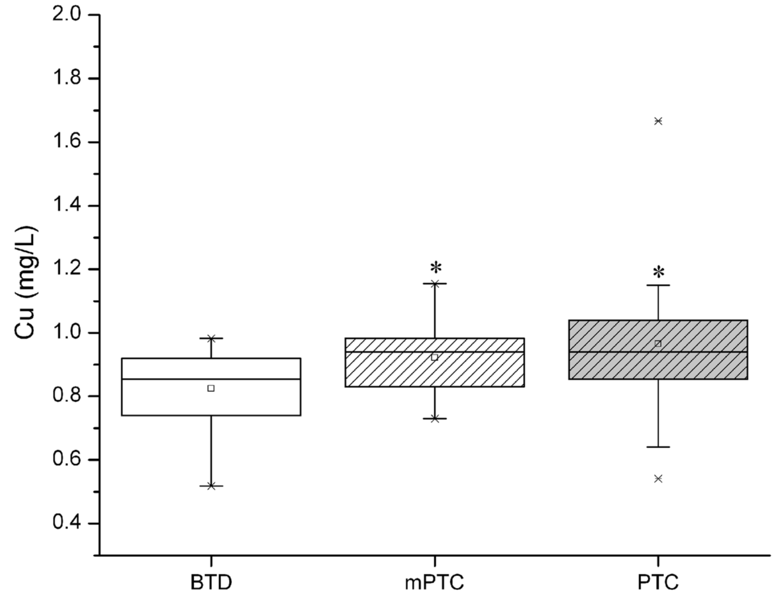

Fig. 1 The concentrations of Cu ions in serum of PTC, mPTC, and BTD patients. Statistically significant at *$p<0.05$

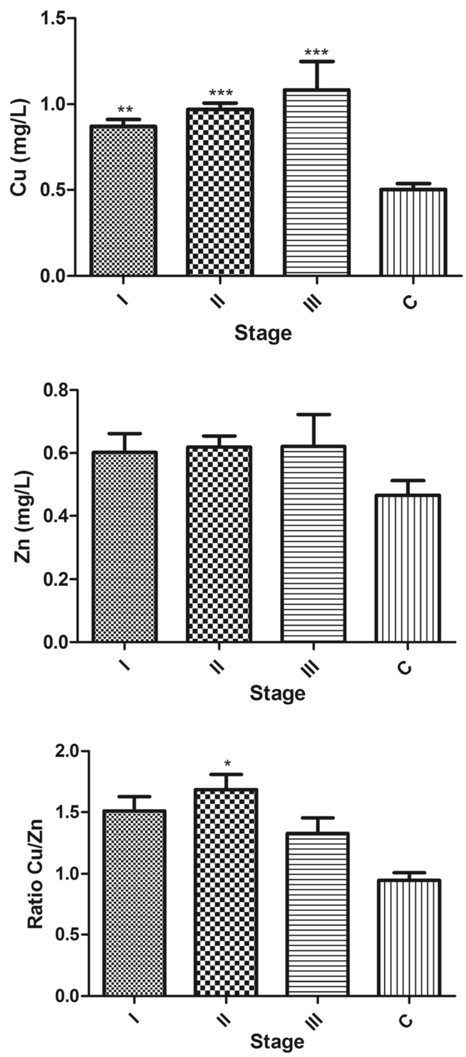

Fig. 2 The concentrations of Cu and Zn ions and Cu/Zn ratio in the serum of patients with different tumor stages and the control group. Statistically significant at *$p<0.05$, **$p<0.01$, and ***$p<0.001$

Cu-transport protein, ceruloplasmin, into serum. This was accompanied by a concerted reduction in mRNA levels of the other Cu-containing proteins such as metallothioneins 1 and 2 or superoxide dismutase 1.

The Cu concentrations were also investigated in the bladder cancer tissue, where it reached higher values in comparison with healthy controls. The Zn levels in the sera and bladder tissue of patients with carcinoma of the bladder were substantially lower as compared to those of the control group. The serum Cu/Zn ratio was higher in the bladder cancer group with tendency to rise in patients with muscle-invasive neoplasm [28].

As thyroid hormones influence the metabolism of trace elements including Cu, some authors studied changes in serum Cu levels in thyroid cancer [29]. According to those, pre- and postoperative serum Cu levels were significantly higher when compared to control group. The postoperative serum Cu levels were significantly decreased compared to those of preoperative in the BTD group. It is suggested that this may be used to demonstrate successful cancer surgery and may have implications for a long-term follow-up of thyroid cancer patient [30].

In our study, the concentration of Cu ions was statistically significantly higher in serum of PTC and mPTC patients than in BTD patients. The concentration of Zn and the Cu/Zn ratio appeared to be of no statistical significance.

Adequate preoperative diagnosis of the patients who require surgery is essential for surgical strategy, i.e., extent of surgery. Preoperative diagnostic tools vary from ultrasonogaphy, scintigraphy, fine-needle aspiration biopsy (FNAB), real-time ultrasound elastography to numerous genetic investigations (BRAF), etc. [1, 31]. All these procedures are usually available in tertiary medical institutions and are connected with high cost-benefit ratio.

According to our study, Cu serum level was statistically higher in PTC and mPTC patients compared with BTD patients and with control group. Therefore, Cu concentration study can become a useful and easy applicabe tool in preoperative evaluation of patients with thyroid diseases which require surgery.

Conclusion

The concentration of Cu ions in serum of patients before thyroid surgery can be useful, available, and a low-cost tool in prediction of preoperatively undiagnosed PTC in patients with BTD.

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Conflict of Interests The authors declare that they have no competing interests.

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