A Comparison of Serum Copper Levels in Patients with Papillary Thyroid Carcinoma, Nodular Goiter, and Healthy Volunteers

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ABSTRACT

Objective: Serum copper (Cu) is an essential trace element that plays a key role in thyroid hormone production. An inappropriate level of serum Cu might be related to development of both benign and malignant thyroid neoplasm. Nodular goiter and papillary thyroid carcinoma (PTC) are common benign and malignant tumors of the thyroid, respectively. This study aims to compare the serum Cu levels of healthy women with women with PTC or nodular goiter.

Materials and Methods: A total of 205 Thai women were recruited for this cross-sectional study. The reference group was comprised of 100 healthy volunteers. There were 61 nodular goiter and 41 PTC patients that had been treated with surgery. Serum Cu was measured using an atomic absorption spectrophotometer and the three groups were compared.

Results: The serum Cu levels of the PTC, nodular goiter and the reference group were 0.93 (0.85, 1.11) µg/ml, 1.03 (0.90, 1.14) µg/ml and 0.97 (0.80, 1.11) µg/ml, respectively. The results were not statistically different ($P = 0.10$). A post hoc subgroup analysis in the PTC group showed only serum Cu levels were significantly higher in the blood vessel invasion group ($P = 0.02$).

Conclusion: The serum Cu levels of patients with PTC and nodular goiter were not different and did not differ significantly from the reference group. Despite related to with only one pattern of histopathologically aggressive PTC-Blood vessel invasion, serum Cu levels cannot be used as an assistive tool for diagnosis and the prognosis of PTC.

Keywords: Serum copper (Cu); nodular goiter; papillary thyroid carcinoma; thyroid cancer; BRAF mutation (Siriraj Med J 2023; 75: 38-45)

INTRODUCTION

Serum Cu is an arbitrary marker for many types of malignancies and is more available and less expensive than genetic testing. Serum Cu levels rise significantly in many types of malignancies, such as esophageal cancer, gynecologic cancer, pancreatic cancer, and melanoma.[1-4] Why serum Cu is elevated in the presence of malignancy is not yet clear. Copper may be a key factor in tumor angiogenesis.[5,6] Ceruloplasmin, the Cu-binding protein, can increase in malignancies due to decreased metabolism

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or an inflammatory response state.[7,8] Zhu et al. found that Cu transportation in tumor cells increased as a result of elevated expressions of transporter genes.[9] Coates and coauthors reported that the sensitivity and specificity of high serum copper for the risk of developing a cancer were 40% and 80.4%, respectively, but a cutoff value was not determined.[10]

Serum copper plays a key role in thyroid hormone production.[11] This trace element controls T4 levels by regulating calcium homeostasis.[12] An inappropriate serum Cu level also stimulates the growth of transformed cell by providing energy (ATP) in cell cycle process.[13] Furthermore, Cu is an essential nutrient that be integrated in the antioxidant process as a cofactor of the enzyme superoxide dismutase, eliminating free radicals caused by various tissue damage in the body.[14] However, excessively high Cu levels can cause abnormal cell growth by creating free radicals and damaging DNA.[15,16]

Several studies have reported that the serum Cu levels of patients with thyroid carcinoma were higher than those of normal subjects.[17-20] Additionally, Baltaci et al. also found that serum Cu decreased after removal of thyroid tumors.[21] In contrast, Al-Sayer et al. did not identify a difference between the serum Cu levels of patients with thyroid cancer and healthy controls, and serum Cu increased after thyroidectomy.[22] Due to insufficient data and inconclusive evidence, the association between serum copper and thyroid cancer needs further investigation. Therefore, we aimed to compare the serum copper levels of patients with PTC, nodular goiter and healthy Thai women.

MATERIALS AND METHODS

Study design

This cross-sectional study was conducted at the Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, from July 2018 to June 2021. Serum Cu was measured by the Clinical Toxicology Laboratory of the Faculty of Medicine Siriraj Hospital. The laboratory has been certified to the ISO 15189 accreditation standard since 2013. This study was approved by the Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. Si 367/2017) and was conducted in accordance with the Declaration of Helsinki.

Study population

Diseases of the thyroid gland are generally more common in females than males. In the Thai population, the female to male ratio of incidence of thyroid cancer is approximately 4.3:1, and the gender ratio of thyroid surgery was 5.2:1 during 2018-2020.[23] Because serum Cu levels can vary by gender,[24] we investigated only female patients. The sample size calculation was based on the primary assumption of differences between serum Cu in thyroid disease and the normal population. Totally, we aimed to collect data from 100 patients with thyroid disease (PTC and nodular goiter) and 100 normal controls. The thyroid disease group was consisted of Thai women aged 18 years and older who had thyroid nodules. Fine needle aspiration was performed prior to surgery. All patients with pathological reports of PTC or nodular goiter who needed surgical treatment for thyroid diseases were eligible. Patients with incidental papillary microcarcinoma, thyroid carcinoma other than PTC, or other thyroid or systemic diseases that could alter serum Cu levels (i.e., thyroiditis, Wilson's disease, pulmonary disease, cardiovascular disease, infectious disease, and other types of cancer) were excluded. Patients with current medications or supplements that would alter serum Cu levels, a history of previous thyroid surgery, or abnormal levels of FT4 or TSH were also excluded. The reference group consisted of healthy female volunteers with normal thyroid glands confirmed by ultrasonography and blood tests showing FT4, TSH, Cr, and eGFR within normal limits. All study subjects were fully informed about the treatment options and study protocol before signing informed-consent forms.

Data collection

Demographic data and ultrasonographic findings of the thyroid gland were recorded. Blood samples were tested for FT4, TSH, Cr, eGFR, and serum Cu levels. For the cancer group, a pathology-confirmed specimen was sent for detection of the $BRAF^{V600E}$ mutation using the PCR-based Sanger sequencing technique combined with allele-specific, real-time PCR. Adverse features such as multifocality, blood vessel invasion, capsular invasion, extrathyroidal extension and evidence of transformation from coexisting nodular goiter were noted. The maximum diameter of the tumors was recorded in centimeters. The risk of recurrence was classified according to the 2015 guidelines of the American Thyroid Association.[25] Stage was classified using the 8th edition of the AJCC/TNM staging system of thyroid cancer.[26] If indicated, post-treatment I-131 total body scans and serum thyroglobulin (Tg) levels were used to detect residual diseases and distant metastases.

Serum Cu level analysis

Blood samples 5 ml were collected with the standard method in accordance with the Clinical and Laboratory Standards Institute guidelines for trace element analysis.[27]

Collection and access procedures were performed in the patient ward one day before surgery with talc-free gloves, a 21-gauge needle, and a BD Vacutainer plastic blood collection tube for trace element testing (K2EDTA). The tubes were kept upright and either immediately sent to the Clinical Toxicology Laboratory or stored in a refrigerator at 2° to 8° C for no longer than 24 hours. The samples were prepared by centrifugation process (3500 round per minute) for 10 minutes then the extracted plasma 0.5 ml was collected and diluted with deionized water 1 ml (1:2). Before analysis, internal quality assurance for trace elements was routinely performed using Clin Check Controls. Next, the sample was analyzed with a flame atomic absorption spectrophotometer to produce free atoms of Cu in the gaseous state. The absorbance of light with the specific wavelength of Cu was measured. The intensity of the absorbed light wave was proportional to the amount of copper in the sample. Subsequently, a standard calibration curve was plotted with linear regression. Serum Cu levels were reported as mg/dl and converted to µg/ml as a standard unit.

Statistical analysis

Demographic data are presented using descriptive statistics. One-way analysis of variance was used to compare the three groups (Reference, PTC, and nodular goiter). If the *P* values were less than 0.05, post hoc analysis was applied. Serum Cu levels (µg/ml) are reported as median and interquartile range. Subgroup analyses of the serum Cu levels of the PTC group were performed for histopathological aggressiveness and $BRAF^{V600E}$ mutation using the Mann-Whitney U test. Variant of PTC, risk of recurrence and the TNM staging were compared by one-way analysis of variance. Pearson's correlation was used to test the association between the size of the PTC or nodular goiter and the serum Cu level. A *P* value of < 0.05 was considered statistically significant. Statistical analyses were carried out using PASW Statistics for Windows (version 18; SPSS Inc., Chicago, IL, USA).

RESULTS

Two hundred and twenty-two subjects were enrolled and 17 were later excluded. The excluded subjects comprised 10 cases with papillary thyroid microcarcinoma, five cases with follicular thyroid carcinoma, and two cases with thyroiditis. Therefore, the study population was 205 subjects, consisting of 105 patients with thyroid disease (44 with PTC and 61 with nodular goiter), and 100 healthy controls. The unequal distribution between PTC (n=44) and nodular goiter (n=61) was due to the enrollment nature of our cross-sectional study design that aimed to recruit consecutive cases and could not preoperatively predict the pathological results of patients.

Serum Cu levels

The serum Cu levels of the PTC group, the nodular goiter group and the reference group, were 0.93 (0.85, 1.11) µg/ml, 1.03 (0.90, 1.14) µg/ml and 0.97 (0.80, 1.11) µg/ml, respectively (Fig 1). The results were not statistically different ($P = 0.10$). Age, serum creatinine and eGFR were significantly different among the PTC, nodular goiter and reference groups (Table 1). After adjustment for age, serum creatinine and eGFR, there was still no statistical difference.

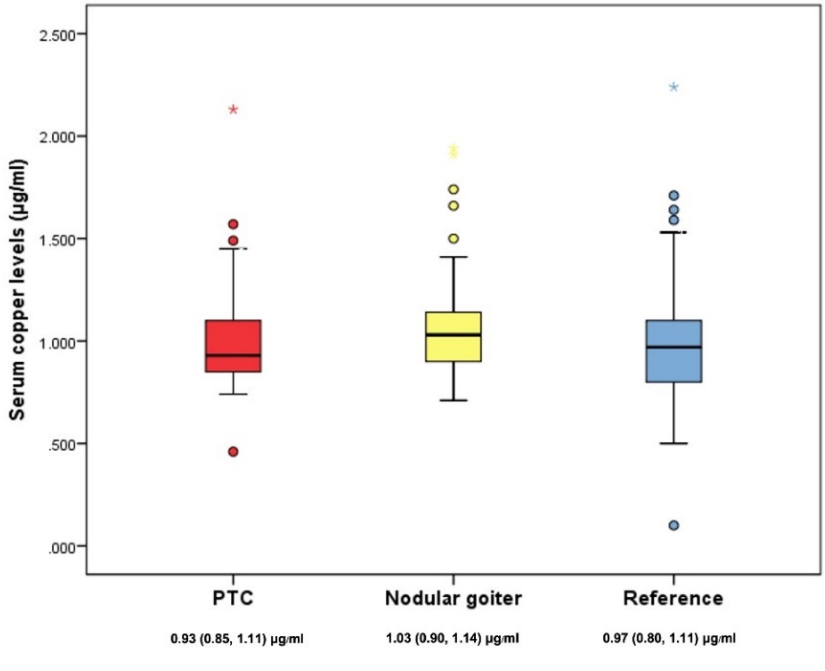

Fig 1. The serum Cu levels of the PTC group, nodular goiter group and the reference group were 0.93 (0.85, 1.11) µg/ml, 1.03 (0.90, 1.14) µg/ml and 0.97 (0.80, 1.11) µg/ml, respectively

TABLE 1. Demographic data and characteristics of PTC, nodular goiter, and the reference group.

Patient characteristics Median (IQR25,75)	PTC (n = 44)	Nodular goiter (n = 61)	Reference (n = 100)	*P* value
Age (years)	45.00 (36.25, 56.25)	48.00 (36.00, 58.00)	37.00 (29.00, 51.00)	0.001*,a,b
Weight (kg)	57.55 (53.20, 68.80)	58.00 (50.00, 66.00)	54.00 (50.00, 65.00)	0.47
Height (cm)	157.00 (152.00, 161.50)	155.00 (153.00, 160.00)	156.00 (153.00, 160.00)	0.83
FT4 (ng/dL)	1.21 (1.08, 1.35)	1.20 (1.10, 1.29)	1.20 (1.08, 1.29)	0.42
TSH (uIU/mL)	1.44 (0.91, 2.76)	1.28 (0.80, 1.92)	1.79 (1.28, 2.49)	0.14
Cr (mg/dL)	0.71 (0.67, 0.83)	0.68 (0.60, 0.77)	0.67 (0.60, 0.74)	< 0.001*,a,c
eGFR(L/min/1.73m^2)	96.54 (86.08, 108.51)	101.18 (89.74, 110.12)	109.07 (99.94, 120.34)	< 0.001*,a,b

* *P* values less than .05 considered statistically significant.

[a] *P* value between reference and PTC was < 0.05

[b] *P* value between reference and nodular goiter was < 0.05

[c] *P* value between PTC and nodular goiter was < 0.05

Abbreviations: PTC, papillary thyroid carcinoma; FT4, free thyroxine; TSH, thyroid stimulating hormone; Cr, creatinine; eGFR, estimated glomerular filtration rate.

PTC group

Histopathological aggressiveness

Of the 44 PTC cases, the classical variant was found in 88.6% (n = 39) and the follicular variant in 6.8% (n = 3). Two patients (4.5%) had non-invasive encapsulated follicular variant of papillary thyroid carcinoma (NIFTP). Multifocal cancers were identified in 70.5% (n = 31) of cases with no statistical difference (*P* = 0.24). The serum Cu in the blood vessel invasion group was significantly higher than those without invasion (Table 2). There were no significant differences in serum Cu levels for coexisting nodular goiter, capsular invasion, extrathyroidal extension, high- risk of recurrence and TNM staging. There was no correlation between tumor size and serum Cu levels (*r* =-0.04; *P* = 0.81). Four patients (9.1%) had distant metastases detected by I-131 total body scan. Their serum Cu levels were higher than those in the no metastasis group, but no significant difference of 1.05 (0.91, 1.38) µg/ml, and 0.93 (0.84, 1.08) µg/ml, respectively (*P* = 0.26). Meanwhile, there was also no significant difference of serum Cu levels among the subgroup of PTC histopathological aggressiveness, nodular goiter and healthy groups.

Molecular testing

The BRAF^{V600E} mutation was positive in 20 patients (45.5%) and negative in 24 (54.5%). The serum Cu levels of BRAF-positive cases were not significantly higher than in the negative groups. 1.02 (0.86, 1.17) µg/ml, and 0.92 (0.83, 0.97) µg/ml, respectively; *P* = 0.06). Nevertheless, the results from both groups remained within the reference range.

Nodular goiter group

The correlation coefficient between the diameters of the nodular goiter and serum Cu levels was (*r*= 0.02; *P* = 0.89). The median serum Cu level in cases of PTC with nodular goiter was 0.92 (0.87, 1.10) µg/ml (n = 17), while the median serum Cu level for cases with pure nodular goiter was 1.03 (0.90, 1.14) µg/ml (n = 61) (*P* = 0.76).

DISCUSSION

We compared serum Cu levels of patients with PTC, nodular goiter and normal healthy subjects. Zhang[24] and Shen[12] reported that gender and ethnicity influence serum Cu levels and so we investigated only Thai women, the

TABLE 2. Comparison of the histopathological, molecular status, risk of recurrence and staging of 44 PTC cases and their serum copper levels.

Histopathological aggressiveness		n (%)	Serum copper level (µg/ml)	*P* value
Coexisting nodular goiter	Yes	17 (38.6)	0.92 (0.87, 1.10)	0.77
	No	27 (61.4)	0.93 (0.84, 1.12)	
Multifocal (Foci > 1)	Yes	31 (70.5)	0.93 (0.85, 1.07)	0.24
	No	13 (29.5)	0.93 (0.83, 1.17)	
Blood vessel invasion	Yes	26 (59.1)	1.01 (0.87, 1.16)	0.02*
	No	18 (40.9)	0.89 (0.82, 0.96)	
Capsular invasion	Yes	25 (56.8)	0.93 (0.85, 1.16)	0.484
	No	19 (43.2)	0.92 (0.85, 1.04)	
Extrathyroidal extension	Yes	20 (45.5)	0.92 (0.84, 1.04)	0.289
	No	24 (54.5)	0.95 (0.86, 1.17)	
Variant	Classic	39 (88.6)	0.93 (0.85, 1.12)	0.50
	Follicular	3 (6.8)	0.92 (0.87, 1.17)[a]	
	NIFTP	2 (4.5)	0.84 (0.75, 0.92)[a]	
$BRAF^{V600E}$ mutation	Yes	20 (45.5)	1.02 (0.86, 1.17)	0.06
	No	24 (54.5)	0.92 (0.83, 0.97)	
Risk of recurrence	Low	11 (25)	0.92 (0.85, 1.04)	0.89
	Intermediate	23 (52.3)	0.93 (0.82, 1.15)	
	High	10 (22.7)	0.92 (0.86, 1.24)	
Tumor (T)	T1	15 (34.1)	0.92 (0.84, 1.04)	0.59
	T2	14 (31.8)	0.95 (0.86, 1.15)	
	T3	12 (27.3)	0.99 (0.83, 1.17)	
	T4	3 (6.8)	0.87 (0.86, 0.88)[a]	
Node (N)	N0	21 (47.7)	0.93 (0.86, 1.06)	0.99
	N1	23 (52.3)	0.93 (0.83, 1.14)	
Metastasis	M0	40 (90.9)	0.93 (0.84, 1.08)	0.26
	M1	4 (9.1)	1.05 (0.91, 1.38)	
Stage	1	34 (77.3)	0.92 (0.84, 1.03)	0.76
	2	7 (15.9)	1.08 (0.91, 1.15)	
	3	1 (2.3)	1.00†	
	4	2 (4.5)	1.19 (0.92, 1.45)[a]	

* *P* values less than .05 considered statistically significant.

† No min, max as there was only one data item for that category.

[a] Use min, max instead of interquartile range.

Abbreviations: NIFTP, Noninvasive follicular thyroid neoplasm with papillary-like nuclear features

gender most often affected by this disease. The median serum Cu levels of our reference group were comparable to those in diverse populations (Table 3). The most common method to measured serum Cu was the atomic absorption spectrometry, so the median serum Cu levels in healthy subjects of our study were very close to the mean value of the studies that used this technique as well as the biggest dataset in a Chinese population.[20,24,28] The results in the reference group showed reliable; However, we did not identify any statistically significant differences in the serum Cu levels among the PTC, nodular goiter, and reference groups.

The use of Cu to support the diagnosis of thyroid cancer remains inconclusive. Baltaci et al.[21] showed that serum Cu levels of women with thyroid cancer were significantly higher than those of healthy controls. Additionally, the serum Cu levels of female patients with thyroid cancer significantly decreased to levels close to those of the control group within two weeks after surgery. Vesna and colleagues[17] compared 35 cases of PTC and 13 cases of papillary thyroid microcarcinoma with 82 cases of benign thyroid tumor. The serum Cu levels of patients with PTC and microcarcinoma were significantly higher than patients with benign thyroid tumor. However, because incidental microcarcinoma was included in the PTC group, their findings are challenging to interpret and to compare with our study.

In 2015, Shen and colleagues published a meta-analysis of five case-control studies investigating serum Cu levels.[12] One study was carried out in China, three in Poland, and one in Turkey. Overall, patients with thyroid cancer had higher serum Cu levels than healthy controls. However, consistent with our results, the Polish studies did not find higher serum Cu levels in patients with thyroid cancer relative to their controls. A 2004 study from Kuwait also reported that serum Cu levels in thyroid cancer patients were not different from healthy controls and rose significantly after thyroidectomy.[22] Hence, ethnicity can influence serum Cu levels. Normally, Cu is actively recycled in the digestive tract, body fluids and tissues, and is mainly excreted from the body via bile. Copper levels are primarily controlled by recycling and resorption, and dietary Cu represents only a small proportion of total Cu resorption.[15] Therefore, dietary intake of Cu has an insignificant effect on serum Cu levels and does not need to be controlled.

In the post hoc subgroup analysis of PTC, we found significantly higher serum Cu levels in patients with blood vessel invasion. Cu is postulated to be a potent stimulator of tumor growth through its activation of angiogenic factors.[29] Nevertheless, the median serum Cu levels were not statistically significant in the presence of adverse features such as positive capsular invasion, extrathyroidal extension, lymph node involvement, distant metastases and high stage. Although, this incidental finding is less likely to demonstrate a relationship between serum Cu levels and the aggressiveness of PTC, the association between serum Cu levels and angiogenesis in thyroid cancer requires further exploration. Furthermore, the additional comparison of serum Cu in each subgroup of

TABLE 3. Serum copper levels in healthy subjects.

Studies	Year	Country	Measurement technique	N	Sex	Serum copper levels (mean ± SD; µg/ml)
Maneeprasopchoke et al.	2022	Thailand	AAS	100	Female	0.97 (0.80, 1.11) †
Zhang et al.[24]	2009	China	AAS	890	Female	1.01 ± 0.24
Baltaci et al.[21]	2017	Turkey	AES	15	Female	0.74 ± 0.24
Przybylik-Mazurek et al.[28]	2011	Poland	AAS	20	All	1.11 ± 0.19
Kosova et al.[20]	2012	Turkey	AAS	37	All	1.06 ± 0.11
Leung et al.[19]	1996	China	AES	50	All	0.74 ± 0.19
Kucharzewski et al.[18]	2003	Poland	TRXRF	50	All	0.69 ± 0.06

† Median and interquartile range

Abbreviations: AAS, atomic absorption spectrometry; AES, atomic emission spectrometry; TRXRF, total reflection fluorescence

PTC with nodular goiter to that of the healthy population showed no significant differences. Thus, we cannot infer that high serum Cu can be used to prognosticate the invasiveness of PTC.

The potential relationship between serum Cu levels and gene mutation in humans has not been studied. Since copper regulates the function of follicular cells, aberrant levels of serum Cu may be associated with molecular alterations. Currently, there are several genetic mutations reported in thyroid cancer and the $BRAF^{V600E}$ mutation is the most common biomarker for PTC. Brady et al. demonstrated that Cu is required for BRAF signaling and tumorigenesis. A reduction in serum Cu levels caused the size of $BRAF^{V600E}$-driven melanomas to decrease in laboratory animals.[30] A recent investigation by Baldari et al. also found that Cu-chelating agents reduced the proliferation, survival, and migration of human colon cancer cells carrying the $BRAF^{V600E}$ mutation.[31] We hypothesized that in thyroid cancer, the $BRAF^{V600E}$ mutation would be associated with increased serum Cu levels, as is seen in melanoma and colon cancer. Besides, no significant elevation of serum Cu levels was observed in PTC with $BRAF^{V600E}$ mutation, suggesting that serum copper does not indicate the severity of PTC.

To our knowledge, this is the first study to report serum Cu levels in terms of histopathological aggressiveness, risk of recurrence, staging, and molecular status in PTC. In addition, we screened all healthy subjects with ultrasonography of the thyroid gland to avoid unexpected thyroid nodules in the control group. This ensured that the reference serum Cu values of the healthy Thai women were reliable and could be used as a standard for further studies. On the other hand, our analyses suggest that serum Cu levels are not appropriate for diagnostic and the prognosis of PTC.

Our study has some limitations. Our subjects were Thai women with PTC and nodular goiter. We did not address the role of serum Cu levels in men, other types of thyroid cancer, and in advanced-stage thyroid cancers such as tracheal or recurrent laryngeal nerve invasion. In addition to Cu, other essential trace elements such as selenium, cadmium, zinc were likely involved in the carcinogenesis of thyroid.[32] The expand study of multiple trace element levels and their ratios would give more informative data about the relation between trace elements and thyroid cancer.

CONCLUSION

The role of serum Cu in the pathogenesis and prognosis of thyroid tumors remains unclear. Serum Cu levels in patients with PTC and nodular goiter were not different, and also were not different from the reference group. However, serum Cu was associated with only one pattern of histopathologically aggressive PTC- Blood vessel invasion. Therefore, serum Cu levels cannot be used as an assistive tool for diagnosis and the prognosis of PTC.

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