

CLINICAL STUDY

Serum copper levels in benign and malignant thyroid diseases

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Abstract: *Objective:* To examine the changes in serum copper (Cu) levels in benign and malignant thyroid disease in humans.

Background: Thyroid hormones influence the metabolism of trace elements including copper.

Methods: 47 papillary thyroid cancer and 43 benign multinodular goitre patients who underwent total thyroidectomy and 37 healthy control subjects were included into this study. All of the patients and controls were females. Serum Cu levels were detected with atomic absorption spectrophotometer.

Results: In the papillary thyroid cancer group serum level of Cu was 131.61±33.9 µg/dL before surgery and 120.81±30.4 µg/dL after 20 days from surgery. In the benign group serum Cu level was 84.75±12.1 µg/dL and 68.01±9.4 µg/dL postoperatively. These results were compared to healthy control's value of 105.87±10.68 µg/dL. In the papillary thyroid cancer group pre- and postoperative serum Cu level was significantly higher when compared to control group ($p < 0.05$). Postoperative serum Cu level significantly decreased when compared to pre-operative level ($p < 0.05$), in which, it was still higher than the control ($p < 0.05$). In the benign group pre- and postoperative serum Cu level was significantly lower than in the control group ($p < 0.05$). Postoperative serum Cu level significantly decreased when compared to pre-operative level in the benign group ($p < 0.05$).

Conclusion: This is a pioneer study to examine serum Cu level in benign and malignant thyroid patients compared to controls. In our small groups serum Cu levels increased in malignant thyroid patients and decreased in the benign group (Tab. 1, Ref. 18). Full Text in PDF www.elis.sk.

Key words: copper, papillary cancer, thyroid cancer, multinodular goitre.

Trace elements are essential micro-nutrients both for humans and other organisms. They are crucial for many physiological processes and are involved in many pathologic changes in tissues (1). Thyroid hormones influence the metabolism of trace elements including copper (Cu) (2). Cu is important for the activity of the enzyme superoxide dismutase (SOD). Changes in the activity of this enzyme are consistent with the Cu serum levels (3). Cu was shown to be essential for cell division both in normal and cancerous tissue (4, 5). Although copper is an essential element for human and animals, a high concentration of Cu (above normal) could induce growth proliferation and cancer by damaging DNA with toxic free hydroxyl radicals (6).

The present study was undertaken to investigate correlation of Cu serum levels in benign and malignant thyroid diseases before and after surgery compared to controls.

Materials and methods

This is an age- and sex-matched case-control study, conducted at the Department of Medical Biochemistry of Celal Bayar University.

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sity of Medicine and the Department of General Surgery of Ankara Oncology Hospital, Ankara, Turkey. The study was approved by the hospital Ethical committee. All the patients and the volunteers involved in the study have given informed consent.

Patients

Recruitment of subjects was performed by convenience sampling at outpatient General Surgery Clinic of Oncology Hospital, Turkey by trained physicians. 47 papillary thyroid cancer patients, 43 benign multinodular goitre patients and 37 healthy control subjects with normal thyroid function tests were included in this study. These patients were female gender presented with multinodular goiter in thyroid ultrasonography and were grouped as malignant and benign after total thyroidectomy according to pathologic examination. Occult papillary carcinoma was excluded from the study. As patients with follicular carcinoma and medullary carcinoma of the thyroid were rare, for statistical analysis only patients with papillary morphology were included into the study. Total thyroidectomy was performed after which they came to a control visit on the twentieth postoperative day and they were not on the replacement therapy. The controls were age and sex matched healthy volunteers.

Assay

Blood samples were kept at -70°C until analysis. All samples from each patient were run in the same assay.

Serum thyroid hormones were measured by radioimmunoassay kits (TOSOH).

For serum levels of Cu, serum is deproteinized with trichloroacetic acid and the supernatant analyzed by atomic absorption spectrophotometer at a wavelength of 324.7 nm respectively, according to the method of Unicam Atomic Absorption Spectrophotometry manual.

Statistics

Non-parametric methods were used in the cross-sectional analysis of biomedical data (Mann-Whitney U test). Two-tailed probability (p) values were calculated and statistical significance was defined as $p < 0.05$. All analyses were performed by statistical software SPSS 10.0.

Results

47 papillary thyroid cancer (mean age 41 ± 13 years) and 43 benign multinodular goitre patients (mean age 39 ± 14 years) who had undergone total thyroidectomy and 37 healthy control subjects (mean age 42 ± 13 years) were included in this study. All of the patients and controls were female. Age, gender (all of the subjects were female), menopausal status were not significantly different between the benign and malignant thyroid patients and the control group. The thyroid hormone levels were within normal limits in all groups. No patient in the study had radiologically and clinically pathologic lymph nodes and neck dissection was not performed. And there was no patient who had got metastatic lymph node which was reported pathologically after total thyroidectomy.

The serum copper level in healthy control group was 105.87 ± 10.68 $\mu\text{g/dL}$ and 131.61 ± 33.9 $\mu\text{g/dL}$ and 120.81 ± 30.4 $\mu\text{g/dL}$ in thyroid cancer patients pre and post operatively. Cu levels of thyroid cancer patients pre-operatively was found to be higher than those in age-matched controls ($p < 0.05$). Post-operatively Cu levels were also significantly higher compared to control ($p < 0.05$) but significantly lower than pre-operative serum Cu levels indicating a decrease in Cu serum levels after surgery ($p < 0.05$). In the benign group serum pre and post operative Cu levels were 84.75 ± 12.1 $\mu\text{g/dL}$ and 68.01 ± 9.4 $\mu\text{g/dL}$. These results were compared to control value of 105.87 ± 10.68 $\mu\text{g/dL}$. In the benign group pre-and postoperative serum Cu levels were significantly lower than in the control group ($p < 0.05$). Post-operative serum Cu levels significantly decreased compared to those of pre-operative levels for the benign group ($p < 0.05$) (Tab. 1).

Discussion

The concentration of trace elements in the thyroid gland is higher than in other tissues (7) and it has been shown that the thy-

roid hormones do influence the metabolism of these elements (2, 8–10). Furthermore, changes in these elements were associated with development of goiter and other thyroid pathologies including cancer (7). Trace elements are essential micro-nutrients both for humans and other organisms. They are crucial for many physiological processes and are involved in many pathologic changes in tissues (1). On the other hand, it is well known that either an excess or a deficiency of certain trace metals can lead to biological disorders. Uda et al observed that the deficiency or excess of trace elements concentrations in cancerous tissues of different organs may be different (11). In the literature, it is described that trace element concentrations of healthy adult thyroid glands were not dependent on sex or age (12). In our series all of the patients and healthy controls were females and there was no statistical difference between the groups ($p > 0.05$).

Copper being a cofactor of some metalloenzymes is required for the maintenance of optimal health, normal cellular homeostasis, optimum function of the immune system and the structure and function of skeletal, cardiovascular and nervous systems (10). Copper is helping to form haemoglobin in the blood facilitating the absorption and use of iron so that red blood cells can transport oxygen to tissues and assisting in the regulation of blood pressure and heart rate (6).

Cu is essential for the antioxidant function of Cu/Zn-SOD, although it may act as a prooxidant towards lipids. Cu deficiency directly affects the function of cuproproteins with antioxidant functions, such as Cu/Zn-SOD, ceruloplasmin, and metallothionein process, and indirectly affects GSH peroxidase activity (13). As seen Cu is important for the activity of the enzyme superoxide dismutase (SOD). Changes in the activity of this enzyme are consistent with the Cu serum levels (3). It was shown that thyroid hormones influence the metabolism of trace elements including Cu (2). Cu was shown to be essential for cell division both in normal and cancerous tissue (4, 5). In extracellular environment, H_2O_2 reacts with Cu, generating highly reactive hydroxyl radicals, which can react with macromolecules in the vicinity and could cause damage (9). Although copper is an essential element for human and animals, a high concentration of Cu (above normal) could induce growth proliferation and cancer by damaging DNA with toxic free hydroxyl radicals (6).

Thyroid hormones are associated with oxidative and anti-oxidative status of the organism. Depression of metabolism by hypothyroidism has been reported to decrease oxidant production and thus protect tissues against oxidant damage. However, data on the oxidative status of hypothyroidism are limited and controversial (13) Zhang et al suggested that homeostasis of metal ions in both serum and erythrocytes could be more or less influenced by the altered thyroid hormones, and they reported that serum Cu and Zn exhibited significantly positive correlation with T3 and T4 (14). Alturfan et al also found decreased T3, T4 and Cu, Zn levels in MMI-induced hyperthyroid rats (13). These results may illustrate the protective effect of these trace elements as cofactors of antioxidant enzymes in limiting oxidative stress.

The present study was undertaken to investigate any correlation of Cu serum levels in thyroid cancer patients before and after

Tab. 1. Cooper (Cu) levels in thyroid cancer.

Group	Cu Levels ($\mu\text{g/dL}$)
Control	105.87 ± 10.68
Malignant prethyroidectomy	$131.61 \pm 33.9^*$
Malignant postthyroidectomy	$120.81 \pm 30.4^{* \#}$
Benign prethyroidectomy	$84.75 \pm 12.1^*$
Benign postthyroidectomy	$68.01 \pm 9.4^{\#}$

* $p < 0.05$ compared to control group, # $p < 0.05$ compared to prethyroidectomy levels

surgery. In this series Cu serum levels increased significantly in thyroid papillary cancer patients when compared to both normal control group and to post-operative levels. After excision of the cancerous thyroid tissue Cu serum levels dropped significantly but were still higher than in the control group ($p < 0.005$). The patients came to a control visit on the postoperative day 20 and they were not on replacement therapy. The post thyroidectomy Cu level decrease might be influenced by hypothyroidism but it must be remembered that Cu levels were low in benign group and high in the malignant. In spite of the decrease it was still high in the malignant group after thyroidectomy. So decreasing levels could not be attributed only to the hypothyroidism. Postoperative 20 days may not be enough to return to the normal values and long term follow-up is necessary.

The elevation of serum levels of Cu in papillary thyroid cancer patients may be related to disease condition as thyroid hormones influence the metabolism of Cu or elevation of Cu from another causes may be the reason of the thyroid cancer, as in extracellular environment, H_2O_2 reacts with Cu, generating highly reactive hydroxyl radicals, which can react with macromolecules in the vicinity and could cause damage. In this study postoperatively serum levels decreased but were still higher than the normal control which may be much more the indication that serum Cu elevation is a result of the disease condition more than being a reason. Serum levels of Cu were not investigated in occult thyroid carcinoma patients and also in follicular, medullar or anaplastic cancers of the thyroid. Levels of Cu in this patient group might give some idea. Some of the other studies show normal serum Cu levels of thyroid cancer patients and an increase after surgery (15). In our series serum Cu levels of benign thyroid patients were lower than controls both before and after thyroidectomy. Besides post thyroidectomy levels were lower than prethyroidectomy levels for the benign group which could be also suggest that decreased serum Cu levels were a result of the disease condition more than being a reason. In opposite to our results Reddy et al (16) have reported lower levels of Cu in the tissue of cancerous thyroid than in normal thyroid tissue and like our results (our results are reflecting serum levels), much lower levels of Cu in the tissue of thyroid adenoma. Other authors have estimated the concentration rates of Cu/Zn in the cancerous thyroid tumour were significantly higher than in the normal and other thyroid disease (12, 17, 18).

In summary, the increase of serum levels of Cu in cancer patients and low serum levels of Cu in benign thyroid disease may be linked to the disease condition. This is a pioneer study and patient numbers are low to reach any conclusion. Larger series are still needed. Also serum levels of Cu must be still investigated in occult thyroid carcinoma. If our results could be confirmed in further studies, serum Cu levels would be promising in differentiating malignant from benign disease in thyroid patients.

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