ORIGINAL ARTICLE

Relationship of hemoxygenase-1 and prolidase enzyme activity with oxidative stress in papillary thyroid cancer

Terzioglu D[1], Teksoz S[2], Arikan AE[2], Uslu E[1], Yılmaz E[3], Eren B[4]

[1]Department of Medical Biochemistry
[2]Department of General Surgery
[3]Department of Organ Transplantation, HLA Laboratory
Cerrahpaşa Medical Faculty, Istanbul University, Istanbul
[4]Council of Forensic Medicine of Turkey, Bursa Morgue Department, Bursa
Turkey

Abstract

Aim: Recent studies associate thyroid cancer with oxidative stress. We aim to clarify the relation between papillary thyroid cancer, oxidative stress, hemoxygenase-1, prolidase enzymes and investigate the availability of these enzymes as markers for diagnosis, success of treatment, and follow-up.

Methods: Thirty-one patients with papillary thyroid carcinoma and 25 healthy control subjects were included in this study. Hemoxygenase-1, prolidase (oxidant stress indicator), malondialdehyde, protein carbonyl, and superoxide dismutase (an indicator of antioxidant defense system) were measured pre-operatively and 30 days after thyroidectomy.

Results: There was a significant decrease in serum levels of malondialdehyde and superoxide dismutase ($p < 0.001$ for both) after thyroidectomy in papillary thyroid carcinoma group. In addition, there was a significant difference in the post-operative serum levels of prolidase, malondialdehyde, protein carbonyl, and superoxide dismutase between papillary thyroid carcinoma and control groups ($p = 0.024$, $p < 0.001$, $p = 0.002$, and $p = 0.016$, respectively) beside significant difference of malondialdehyde, protein carbonyl, hemoxygenase-1, and superoxide dismutase pre-operative serum levels ($p < 0.001$, $p = 0.003$, $p = 0.006$, and $p = 0.025$, respectively).

Conclusion: When the unquestionable role of oxidative stress in the pathogenesis of cancer is considered, in the future it is expected to associate parametric changes in the serum of patients caused by oxidative stress to papillary thyroid cancer. Hippokratia 2016, 20(1): 55-59

Keywords: Thyroid cancer, antioxidants, superoxide dismutase, malondialdehyde, protein carbonyl

Corresponding Author: Bülent Eren, M.D., Associate Professor, Pathologist, Forensic Medicine Specialist, Council of Forensic Medicine of Turkey, Bursa Morgue Department; 16010, Bursa, Turkey, tel: +902242220347, fax: +902242255170, e-mail: drbulenteren@gmail.com

Introduction

Thyroid carcinomas are the most common type of endocrine cancers. The annual incidence of thyroid cancer varies from 0.5 to 10 per 100,000 in different regions of the world[1]. Thyroid cancer is responsible for 66 % of the deaths related with malignant endocrine neoplasms[2]. The most common thyroid cancer is papillary thyroid cancer (PTC); it forms almost 80 % of thyroid cancers and is higher in women, twice as it is in men[3].

Recent studies associated thyroid cancer with oxidative stress (OS)[1,4,5]. These studies assessed the relationship of various oxidative and antioxidant systems with thyroid cancers and showed the relation of thyroid cancer with oxidative stress.

Oxidative stress which is formed by the breakdown of the balance between free radicals and antioxidants in favor of free radicals, plays a significant role in the pathogenesis of many diseases and mechanisms of complications[6]. It is thought that, reactive oxygen species (ROS), mainly superoxide anion, hydrogen peroxide, and hydroxyl radical, carry a carcinogenic potential and trigger the invasion of cancer cells[4,7].

ROS levels are controlled by antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Malondialdehyde (MDA) is a lipid peroxidation indicator, and its level is mostly used as an indicator of oxidative damage in cells and tissues[4]. The balance between the formation and clearance of lipid peroxides determines the peroxide levels in cells. This balance could be disrupted due to the decrease of cellular defense mechanisms and increase of the peroxidation reactions[8]. Oxidative stress often occurs with H_2O_2 use during thyroxin synthesis in thyroid tissue and ROS production in inflam-

mation and active proliferation phases of tumors[9,10].

Hemoxygenase-1 (HO-1) enzyme is a microsomal enzyme that takes place in the degradation pathway of heme and has a rate limiting function on carbon monoxide production. When the balance of the organism is altered, the activity of HO-1 increases. It was reported that HO-1 acts indirectly as an antioxidant to protect cells and organs[11,12].

Prolidase enzyme (iminopeptidase) is the enzyme that breaks down the iminopeptides, peptides containing proline or hydroxyproline in C terminal. These dipeptides are produced in the organism during collagen breakdown[13]. In literature, elevated serum concentration of prolidase enzyme in cancers was observed, and it was reported that this shows a positive correlation with total oxidant stress[13,14].

According to current knowledge, there is no study in the literature showing the relation of HO-1 and prolidase enzyme with oxidative stress in papillary thyroid cancer patients. The purpose of this study was to investigate the association of HO-1 and prolidase enzymes with oxidative stress and to assess the usability of these enzymes in diagnosis and evaluation of success in the treatment of papillary thyroid carcinoma.

Materials and Methods

Patients

This was an age- and sex-matched case-control study, conducted at Istanbul University, Cerrahpasa Medical Faculty, in the Departments of Medical Biochemistry and General Surgery. Recruitment of patients was performed by convenience sampling at the outpatient clinic of General Surgery. Thirty-one patients and 25 control individuals were included in this study. As follicular thyroid carcinoma cannot be diagnosed by fine needle aspiration (FNA), only the patients with papillary morphology on FNA were included into the study. Individuals who attended the institution's blood bank for blood donation and did not fall in the exclusion criteria were included in the healthy control group. For each thyroid cancer patient, a control patient was matched with same gender and close age.

Individuals with diabetes mellitus, hypertension, on hormonal therapy such as oral contraceptives, thyroxine derivatives, estrogen replacement therapy, and medications for chronic diseases were excluded. Subject were grouped as healthy controls and patients with papillary thyroid cancer.

This study was conducted in accordance to the Declaration of Helsinki, was approved by Istanbul University, Cerrahpasa Medical Faculty, Clinical Research Ethics Board (decision No 29233/26-7-2011), and was funded by Istanbul University Scientific Research Projects (project No 19582). A written informed consent was obtained from each participant.

Assays

Fasting venous blood samples were withdrawn once from the control group and on two occasions from the thyroid cancer patients on the morning of the operation and on the morning of the 30th postoperative day, to eliminate the effect of operative stress on MDA and SOD.

Samples were kept in -80 °C after 10 minutes of centrifugation at 5,000 rpm, until the time of the analysis. All the serum samples were measured for oxidative stress biomarkers simultaneously. Before the measurement of the biomarkers, serum samples were balanced to room temperature avoiding thawing and refreezing[15].

Serum thyroid hormones (Immulite 2000 device, DPC, LA, USA), total cholesterol (Abbott C8000 automatic analyzer, IL, USA), and glucose (Abbott C8000 automatic analyzer, IL, USA) were measured by commercially available enzymatic reagents adapted to an auto analyzer. HO-1, prolidase, MDA, protein carbonyl (PCO), and superoxide dismutase (SOD) (as an indicator of the antioxidant defense system) were measured in taken samples. HO-1 was measured via ELISA method (Eastbiopharm, Hangzhou, China), prolidase with modified photometric method[16], PCO with Levine method[17], SOD with Sun method[18], and MDA with Thiobarbituric acid method[17,19].

Statistical analysis

Data were evaluated using the Statistical Package for Social Sciences (SPSS) software, version 20.0 for Windows (IBM Corp., Armonk, NY, USA). Values were denoted as a mean ± standard deviation. Student's t-test was performed for continuous variables, and p value lower than or equal to 0.05 was accepted as statistically significant.

Results

Preoperative and postoperative measured values of patients and those of control group were compared. Mean age was 49.28 ± 9.52 years for the control group and 50.74 ± 11.18 years for the thyroid cancer group ($p = 0.606$). In the control group 17 (68 %) were females and eight were males (32 %) whereas in the thyroid cancer group 23 (74.2 %) were females and eight (25.8 %) were male ($p = 0.610$). In the thyroid cancer group, fasting plasma glucose (FPG) was preoperatively 104.65 ± 43.62 mg/dL and postoperatively 101.68 ±3 7.20 mg/dL; the other values measured preoperatively and postoperatively were for cholesterol 211.45 ± 58.16 mg/dL and 220.58 ± 60.10 mg/dL, for fT3 3.05 ± 0.55 pg/mL and 2.28 ± 1.209 ng/dL, for fT4 1.13 ± 0.35 ng/dL and 1.07 ± 0.79 ng/dL, and for thyroid-stimulating hormone (TSH) 2.42 ± 4.33 μIU/mL and 25.19 ± 35.80 μIU/mL, respectively. Among these parameters, a significant difference was observed only in FPG, cholesterol, and fT4 levels between before and after the operation ($p < 0.001$, $p < 0.001$, and $p = 0.05$, respectively). No difference was found in fT3 and TSH values ($p = 0.65$ and $p = 0.83$, respectively). MDA was 6.35 ± 1.55 preoperatively and 4.58 ± 1.15 postoperatively in the thyroid cancer patients whereas it was 3.503 ± 0.72 in the control patients ($p < 0.001$). SOD was 1.67 ± 0.55 preoperatively and 0.87 ± 0.75 postoperatively in the thyroid cancer patients whereas it was 1.26 ± 0.89 in the control patients ($p < 0.001$). Detailed values of oxidase enzymes and oxidative stress products (prolidase, MDA, PCO, HO-1, and

Table 1: Differentiation of oxidative enzymes and oxidative stress products before and after the operation in 31 patients with papillary thyroid cancer and 25 healthy controls that were included in this study.

	Prolidase	MDA	PCO	HO-1	SOD
Preoperative	850.81 ± 272.41	6.35 ± 1.55	186.83 ± 51.22	1.61 ± 0.48	1.67 ± 0.55
Postoperative	845.26 ± 195.76	4.58 ± 1.15	176.034 ± 25.46	1.73 ± 0.76	0.87 ± 0.75
p-value	0.936	<0.001	0.293	0.247	<0.001
Control	738.85 ± 131.57	3.503 ± 0.72	150.33 ± 33.49	2.07 ± 0.731	1.26 ± 0.89
Preoperative	850.81 ± 272.41	6.35 ± 1.55	186.83 ± 51.22	1.61 ± 0.48	1.67 ± 0.55
p-value	0.06	<0.001	0.003	0.006	0.025
Control	738.85 ± 131.57	3.503 ± 0.72	150.33 ± 33.49	2.07 ± 0.731	1.26 ± 0.89
Postoperative	845.26 ± 195.76	4.58 ± 1.15	176.034 ± 25.46	1.73 ± 0.76	0.87 ± 0.75
p-value	0.024	<0.001	0.002	0.10	0.016

All values were denoted as mean ± standard deviation, in ng/ml, bold typed p values are statistically meaningful. MDA: malondialdehyde, PCO: protein carbonyl, HO-1: hemoxygenase-1, SOD: superoxide dismutase.

SOD) of both groups are shown in Table 1. No metastasis of PTC was detected in any of the thyroid cancer patients.

Discussion

Thyroid pathologies occur in a wide range from hypothyroidism caused by thyroid gland atrophy to hyperthyroidism due the neoplastic proliferation. The complex and tight interaction of environmental factors such as infections, iodine deficient nutrition, and stress with genetic factors play a role in pathogenesis[5]. The relation of thyroid dysfunction with oxidative stress was shown in experimental animal models and human studies[9,20,21]. In fact, thyroid gland itself is the place where the reactive oxygen molecules are produced with stimulation by TSH. Reactive oxygen molecules are used as substrates for thyroperoxidase enzyme in thyroglobulin iodination and thyroid hormone synthesis[16]. It needs to be investigated if the extrathyroidal situations causing oxidative stress are effective or not in thyroid cancer patients[16].

The study by Wang et al[16] showed the existence of a strong relationship between thyroid cancer and oxidative stress. In recent years, other researchers also reported high oxidative stress as a possible risk factor in the molecular mechanism of thyroid cancer[1,4,22,23].

In this study, serum SOD levels were found to be significantly higher preoperatively than postoperatively in the patient's group and in the control subjects. Sugawara et al[23] and Durak et al[22] detected a significant decrease in the patient's group compared to the control subjects in contrary to Akinci et al[4] and Senthil et al[1] who found that papillary thyroid carcinoma had increased MDA levels, but decreased SOD activity. In contrast, in the current study, similar to the studies of Lassoued et al[5] and Sadani et al[22], a significant postoperative decrease was observed in SOD activity. This decrease suggests that SOD activity has preoperatively been increased as a compensatory response to oxidative stress in PTC patients. Also, a significant reduction of SOD activity was found after thyroidectomy, compared to control individuals. Thus, high levels of SOD activity may evoke suspicion of papillary thyroid cancer. Therefore, it looks like SOD activity plays an important role in papillary thyroid cancer development. Due to the small patient number of this study it is not possible to draw a definitive conclusion on this issue, and further studies with larger patient series are needed.

Akinci et al[4], Mano et al[23], and Sadani et al[22] in their studies found significantly higher concentrations of MDA in PTC than normal thyroid tissues. Similar to these studies, in our study the preoperative serum MDA levels were found statistically significantly higher than the postoperative levels. As we detected statistically significantly higher MDA levels in PTC group even postoperatively compared to the control group, it could be assumed that the increase of MDA levels was derived from extra-thyroidal tissues.

The increase of free radicals in thyroid cancer is explained by the increased lipid peroxidation and damage of the antioxidant system[24]. In this study, an increase of MDA was observed together with an increase of SOD activity which may suggest that the increase in antioxidant enzyme activity is not enough to compensate the lipid peroxidation. Considering the euthyroid status of the study groups, it can be assumed that there could be a shift to the oxidant pathway. As a result, it may be important to evaluate MDA levels after thyroidectomy for the treatment success in PTC. Thus, more studies are needed.

HO-1 is the rate-limiting enzyme in heme catabolism. It was shown that HO-1 is related to cell proliferation and growth. Additionally, HO-1 takes place in the pathogenesis of many cancer types by providing resistance to apoptosis in various cell types[25,26]. In contrary to the study of Chen et al[25] which reported that the increased HO-1 activity decreases the sensitivity to apoptotic stimulations in PTC cells, this study reported slightly lower HO-1 activity in pre- and post-operative PTC patients compared to the control group. Also, HO-1 activity was found higher in the postoperative compared to preoperative period but it was not statistically significant. On the other hand, HO-1 activity preoperatively was found significantly lower than in the control group and this was statistically significant ($p = 0.006$). Based on these results, the HO-1 enzyme deficiency or suppression can be considered to play a role in the pathogenesis of PTC. However, to explain this difference, further studies including apoptotic markers are needed.

Prolidase enzyme is a member of the matrix metalloproteinase family. It plays an important role in the break-

down of collagen, matrix structuring, and cell growth. The activity of prolidase enzyme has been investigated in various cancer types such as pancreatic, lung, gastric, breast, ovarian, and endometrium carcinoma[14,27-31]. In our study, despite the slightly increase detected pre- and postoperatively in PTC group compared to control group, this was not statistically significant. To our knowledge, there is no previous study regarding the serum prolidase activity in PTC. The importance of the present study is reporting these initial research findings on this issue. The reasons we did not find a significant difference might be either that prolidase enzyme activity does not have a role in PTC development or maybe the small patient number included in the study.

Oxidative modifications of enzymes and structural proteins play a major role in the etiology and/or the progression of numerous diseases. Oxidatively modified amino acids and their derivatives are used for screening of protein oxidation, and new ones are being added to the list. PCO content is the most common and useful biomarker of protein oxidation[32]. In this study, preoperative and postoperative values of PCO in the PTC patient group were significantly higher than the control group. However, there was no significant difference between the preoperative and postoperative values. Given the absence of metastasis in patient groups in this study, it could be thought that PCO could be originating from extrathyroidal tissues. Another importance of the present study is being the first study that analyzed serum PCO in PTC.

A limitation related to this study is that the control group did not include any patient with benign thyroid disease. A study comparing benign thyroid disease with papillary thyroid carcinoma in the aspect of oxidative stress could indicate any existing difference.

Conclusion

When the unquestionable role of oxidative stress in the pathogenesis of cancer is considered, in the future, it is expected to associate parametric changes in the serum of patients with alterations caused by oxidative stress. Also, it will make it easier to understand the role of reactive oxygen species in the mechanism of cancer formation and point out the importance of the additional antioxidant treatment in the fight against cancer.

Conflict of interest

Authors declare no conflict of interest.

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