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Tryptophan Degradation and Antioxidant Status in Patients With Thyroid Disorders

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Abstract

Background: The aim of this study was to evaluate the degradation of tryptophan (Trp), neopterin production and antioxidant capacity in patients with benign and malignant thyroid disease.

Methods: For this reason, the levels of tryptophan, kynurenine (Kyn) and neopterin, and superoxide dismutase (SOD) and catalase (CAT) enzyme activities in 67 thyroid patients were evaluated in our study and the results were compared with 30 healthy controls.

Results: Tryptophan and kynurenine levels in thyroid patients decreased compared to the control group. Patients with thyroid disease had lower CAT activity than the control group. The neopterin and tryptophan levels in malignant and benign patients were also significantly different.

Conclusion: The results of the present study suggest that thyroid disorders may lead to changes in tryptophan degradation, neopterin production and CAT enzyme activities.

Keywords: Antioxidant enzymes, Indoleamine 2,3-Dioxygenase activity, Kynurenine, Neopterin, Thyroid diseases, Tryptophan

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Introduction

Thyroid disorders are among the most common diseases worldwide. The prevalence of thyroid cancer is 2.4%, and it is one of the most important of these diseases.[1] There are four different types of thyroid cancer: papillary, follicular, medullary and anaplastic. The most common type is papillary thyroid cancer (PTC; 88%), whereas the rate of follicular type is 9% and the rate of medullary and anaplastic type is 3%.[2-5]

The immune system plays a critical role in the identification and prevention of tumor formation and metastasis. Cancer cells find the opportunity to reproduce and spread when the immune system is depressed or by developing mechanism of evasion of immune surveillance.[6-8] Immune system activation is known to be an important cause of antioxidant consumption and oxidative stress development. This condition further deepens in chronic immune activation.[9-11] In human body, the existence of many defense mechanisms is well known. They are mainly extracellular, membrane bound and intracellular antioxidant defense systems. Enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) are among enzymes involved in the cellular antioxidant defense mechanisms.[12,13] Although the role of the cellular antioxidant defense system in neoplastic diseases is known, the relationship between thyroid cancer and antioxidant defense system is not known precisely.[14] Therefore, it is assumed that measurement of SOD and CAT enzyme activities together with immune system parameters in patients with thyroid cancer will give an idea about the differentiation of different thyroid diseases and the stage of disease.

Indoleamine 2,3-dioxygenase (IDO) is an enzyme secreted from dendritic cells, monocytes, and macrophages, and is induced by Th1-type cytokines such as interferon gamma (IFN-γ) produced particularly by T cells of the innate and acquired immune system.[15-17] IDO-secreting dendritic cells suppress T-cell proliferation by utilizing tryptophan locally or by synthesizing apoptotic kynurenine metabolites.[16]

Neopterin is one of the immune system activation parameters secreted from human monocyte/macrophages via IFN-γ. In malignant diseases, neopterin levels vary depending on tumor type. In the advanced

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stages of tumor progression, neopterin levels are reported to be higher compared to early stages.[18-22] There are very few studies on the relationship between the neopterin levels and thyroid cancer.[18,23-25] The increase in immune-mediated tryptophan degradation results in a decrease in serum tryptophan levels, an increase in kynurenine levels and accordingly an increase in neopterin levels as an indicator of Th1-type immune activation.[26]

In our previous study, it was shown that there were significant differences in urine neopterin levels between patients with malignant and benign thyroid tumors. Also, it was noted that neopterin levels could be beneficial particularly in differentiating tumor types in clinical practice which could be used as a biomarker in the diagnosis of thyroid tumors.[18]

In this study, our aim was to investigate the differentiation of tumor types and their stage by comparing the levels of tryptophan degradation as an indicator of IDO activity, the levels of neopterin as a parameter of cell-mediated immune activation, and the cellular antioxidant enzyme activity in benign and malignant thyroid diseases.

Materials and Methods

Subjects and Samples

This study was conducted with a total of 67 patients (47 females and 20 males) who were admitted to General Surgery Department of Gazi University Medical Faculty with the complaints related to various thyroid diseases and underwent surgery, between January and June 2006. The patients were divided into two groups. A total of 24 patients (17 females, 7 males, mean age: 45.43 ± 2.71) had malignant thyroid disease with PTC. The second group consisted of 43 patients (30 females, 13 males) with benign thyroid diseases, lymphocytic thyroiditis (mean age: 44.39 ± 2.30) and multinodular goiter disease (mean age: 46.23 ± 3.53). In the control group, there were 30 (21 females, 9 males) healthy individuals (mean age: 28.29 ± 0.90) who were staff members in the same hospital and had no malignancy, infection or immunologic abnormality. All of the individuals in the control group were thoroughly questioned whether they were healthy and did not use any medication during the study period.

Blood samples were taken in a non-heparinized vacuum tube in the early morning hours before the surgery and centrifuged at 3.500 rpm. The supernatants were collected and stored at -20°C until measurement.

Determination of Tryptophan Degradation and Kynurenine Concentrations

Serum tryptophan and kynurenine concentrations were measured by high performance liquid chromatography.[27] The ratio of kynurenine to tryptophan (Kyn/Trp) was calculated to determine the degree of tryptophan degradation and IDO activity. The Kyn/Trp ratio was used as an indicator of IDO activity.[28]

Determination of SOD and CAT Activities

Blood samples were taken in heparinized tubes and centrifuged at 3.500 rpm for 15 minutes. The erythrocytes were washed with isotonic phosphate buffer (pH 7.4). The hemolysates were prepared by adding cold deionized water, and the cellular residues were removed by centrifugation. The SOD and CAT enzyme activities were measured by using supernatants.[20] The CAT activity was measured by the method described by Aebi,[29] and the SOD enzyme activity was measured by the Marklund and Marklund method.[30]

Measurement of Neopterin Levels

Blood samples from the patient group and control group were separated into sera and stored at -20°C until the day of study. Serum neopterin levels were measured by enzyme-linked immunosorbent assay (ELISA) method using the Brahms (Hennigsdorf, Germany) brand kit.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software, version 11.5 (SPSS Inc., Chicago, IL, USA). Demographic data were expressed in mean ± standard error of the mean (SEM). Descriptive data were expressed in median and interquartile range (IQR). The Kruskal-Wallis analysis was used in the assessment of the differences between groups, while the Mann-Whitney U test was used in comparison between two independent groups. The correlations were analyzed by non-parametric Spearman's rho (R_s) values. A P value of <0.05 was considered statistically significant.

Results

Fine needle aspiration (FNA) was used in the preoperative diagnosis of cancer patients. Exclusion criteria of the study were as follows: the patients who were not diagnosed with PTC by intraoperative frozen-section (they were diagnosed with PTC by FNA in the preoperative period), the patients in whom permanent histopathological evaluation could not be reached in the postoperative period, those with a history of chemotherapy or radiotherapy, and those with a history of cervical radiotherapy. The patients were staged according to the sixth edition of the cancer staging system developed by the American Joint Committee on Cancer (AJCC).[31]

The study groups consisted of patient group (patients with malignant and benign thyroid diseases) and control group. The parameters of the patient groups and the

control group are presented as median (IQR) error in Table 1.

Tryptophan Degradation, Kynurenine Concentrations and IDO Activity

There was a significant decrease in tryptophan levels in all patients compared to the control group ($P = 0.012$). When tryptophan levels of study groups were compared among themselves, there was a significant decrease in tryptophan levels in patients with malignant tumors compared to the control group ($P = 0.003$), but the decrease in tryptophan levels in patients with benign tumors was not significant ($P = 0.081$). The tryptophan levels in patients with malignant thyroid diseases were lower than those with benign thyroid diseases ($P = 0.001$).

When the concentrations of kynurenine were compared with the control group, there was a significant decrease in all patient groups ($P = 0.003$). There was a significant decrease in the kynurenine levels in both patient groups (benign thyroid diseases $P = 0.022$, malignant thyroid diseases $P = 0.002$) compared to the control group. No significant difference in the level of kynurenine was found between benign and malignant thyroid patients ($P = 0.212$).

Changes in IDO activity, calculated as the ratio of kynurenine to tryptophan, were not statistically significant when compared to the control group ($P = 0.763$) and also when differences between groups were assessed. There were no significant differences in IDO activity of both patient groups (benign thyroid diseases $P = 0.851$, malignant thyroid diseases $P = 0.696$) compared to the control group, and also between benign and malignant patients ($P = 0.771$).

SOD and CAT Activities

Although the SOD activity in the patients groups was higher than the control group, there was no difference between two studied groups in case of SOD activity ($P = 0.421$). Also, there were no significant differences in the SOD activity in benign thyroid patients ($P = 0.408$) and malignant thyroid patients ($P = 0.633$) compared to the control group, and also between benign and malignant groups ($P = 0.779$).

The CAT activity in the patient group was lower than the control group ($P < 0.001$). The change in CAT activity in patients with malignant and benign thyroid disease was significant compared to the control group ($P < 0.001$). However, no significant difference was found between the patient groups ($P = 0.529$).

Neopterin Levels

Compared to the control group, there was a decrease in neopterin levels in all patients and also in benign group, but there was an increase in malignant group; however, none of these changes were statistically different ($P = 0.655$). The neopterin levels in patients with malignant ($P = 0.408$) and benign ($P = 0.249$) thyroid diseases were not significant compared to the control group. On the other hand, the increased levels of neopterin in patients with malignant thyroid diseases were found to be significant in comparison with patients in the benign group ($P = 0.023$).

The relationship between parameters was analyzed by Spearman correlation test. Correlation results are shown in Table 2.

Discussion

A number of changes in cell physiology prepare the ground for the development of malignant tumors. The interactions between the host and environment lead some changes in the normal cell physiology. Immortalization (suppression of apoptosis mechanisms), gaining autonomy in growth signals and insensitivity (or resistance) to anti-growth signals are among the specific features of cancer cells. In addition, other features that emerge as a result of interaction with the host include increased proangiogenic activity, invasion, metastasis and immunological escape.[6]

In vivo, the increase in cytokine-induced tryptophan degradation is observed under conditions in which the cellular immune response is induced. In this case, an increase in kynurenine and/or other tryptophan degradation products may be observed together with a decrease in serum tryptophan concentrations.[32] To

Table 1. The Levels of Pteridine Pathway Components and Antioxidant Enzyme Activities in the Study Groups

Group	Median (IQR)					
	Neopterin (nmol/L)	Kyn (μmol/L)	Trp (μmol/L)	Kyn/Trp (μmol/mmol)	SOD (IU/g protein)	CAT (IU/g protein)
Control	6.03 (2.59)	1.99 (0.48)	64.99 (20.55)	29.76 (7.75)	3.40 (1.37)	2.26 (0.23)
Thyroid	6.20 (2.82)	1.70 (0.36)[a]	58.38 (9.07)[a]	30.00 (7.89)	3.60 (1.42)	1.10 (0.22)[a]
Malignant	7.30 (2.88)	1.66 (0.25)[a]	55.15 (7.65)[a]	29.35 (8.77)	3.60 (0.94)	1.08 (0.16)[a]
Benign	5.37 (2.34)[b]	1.75 (0.44)[a]	60.62 (9.41)[b]	30.08 (8.26)	3.63 (1.74)	1.11 (0.28)[a]

Abbreviations: Kyn, kynurenine; Trp, tryptophan; IQR, interquartile range; SOD, superoxide dismutase; CAT, catalase.
[a] $P < 0.05$; statistically significant difference with control.
[b] $P < 0.05$; statistically significant difference with malignant thyroid patients.

Table 2. The Correlations Between Parameters

Correlations	Control	Benign	(Rs)		
			Malignant	Patient Groups	All Groups
Kyn-IDO	0.646[b]	0.867[b]	0.866[b]	0.838[b]	0.724[b]
Trp-IDO	-0.416[a]	-0.312[a]	-0.360	-0.340[b]	-0.329[b]
Neop-CAT	0.288	-0.075	0.451[a]	0.036	0.097
Neop-SOD	-0.430[a]	0.068	-0.190	-0.011	-0.095
CAT-SOD	-0.106	-0.404[b]	-0.104	-0.334[b]	-0.276[b]
Trp-Kyn	-0.016	0.158	0.067	0.185	0.251[a]
Trp-CAT	0.088	0.021	-0.080	-0.019	0.231[a]

Abbreviations: Kyn, kynurenine; Trp, tryptophan; IQR, interquartile range; SOD, superoxide dismutase; CAT, catalase, Rs, Spearman's rho value.
[a] $P<0.05$; [b] $P<0.01$.

show that tryptophan degradation is dependent on IDO activation rather than tryptophan 2,3-dioxygenase (TDO), the relationship between the immune system activation parameters and the metabolic pathways of tryptophan should be well known. In cases where IDO is active, there is a correlation between the Kyn/Trp ratio and the endogenous IFN-γ release which is an immune activation parameter. Under normal physiological conditions, kynurenine concentration is related to tryptophan concentration, and a convenient indicator that tryptophan degradation is due to the activation of IDO rather than TDO is to demonstrate concomitant immune system activation. Thus, activated IDO is indicated when kyn/trp correlates with an immune activation parameter such as neopterin. Pro-inflammatory cytokines and IFN-γ limit the utilization of tryptophan in cells by inducing the IDO enzyme in many cells.[15,33] Due to the fact that tryptophan is necessary for protein synthesis, protein biosynthesis and cell growth stop in deficiency of this essential amino acid. Consequently, the depletion of tryptophan may be a defense mechanism induced by IFN-γ in immunocompetent cells during the immune response. The activation of IDO also inhibits the response of T cells to in vitro and in vivo mitogenic stimulation. In addition to inadequate levels of tryptophan, pro-apoptotic tryptophan degradation products such as kynurenine levels are also important in disorders that involve cellular immune activation. In patients with tumor, it is suggested that tryptophan degradation is directly related to the immunological escape mechanism in tumor cells.[15] Reduced tryptophan concentration and increased concentrations of kynurenine and other tryptophan degradation products have been shown in many diseases. It is thought that there is a relationship between the rapid degradation of tryptophan and many malignant diseases such as solid tumors and hematologic neoplasms. Low tryptophan concentration and increased Kyn/Trp ratio also provide information on the stage of the disease.[17,32,34] The relationship between tryptophan and cancer was studied for the first time in the 1950s, and an increase in urinary tryptophan degradation products was reported in patients with bladder cancer.[6] Okamata et al observed that tryptophan degradation is increased due to the induction of IDO by IFN-γ in the CMT-93 cell line of mouse rectal carcinoma.[35] Although the relationship between IDO and thyroid cancer is not well known, excessive secretion of IDO is thought to have an effect on the development and spread of thyroid cancer. In addition, some tryptophan metabolites such as kynurenine induce apoptosis.[36] It has been suggested that increase of IDO in thyroid cancer plays a critical role in immunosuppression of these patients.[37] PTC and benign thyroid nodules are frequently observed among patients. Although the metabolic differences between these two conditions have not yet fully differentiated, common features have been reported as glycosylation, amino acid metabolism, single carbon metabolism and increase in tryptophan metabolism.[38] Under normal conditions, the concentration of kynurenine is dependent on the concentration of tryptophan. When dietary intake of tryptophan declines, there is also a decrease in endogenous tryptophan levels as well as a decrease in concentration of kynurenine. Therefore, instead of measuring concentrations of tryptophan or kynurenine, evaluation of the Kyn/Trp ratio gives more meaningful results.[15,32] In our study, we found a significant decrease in levels of tryptophan and its degradation product (kynurenine) in all patient groups compared to the control group. The reduction of tryptophan levels in thyroid patients may be an IFN-γ-induced defense mechanism. The decrease in kynurenine concentration is due to a decrease in tryptophan concentration. It was found that tryptophan levels were significantly different in patients with malignant and benign thyroid diseases. In the presence of malignancy, by triggering the utilization of tryptophan, its level was lower than the benign patients group. Although the levels of kynurenine decreased in patients with both malignant and benign thyroid diseases,

there was no significant difference between both patient groups compared to the control group.

There is a direct relationship between excessive secretion of IDO and the failure of cancer treatment. In ovarian cancer, a negative correlation was found between excessive secretion of IDO in tumors and chance of survival in patients.[39] Unfortunately, there was no significant difference between groups in Kyn/Trp ratio which is another indicator of IDO activity. This condition may be due to the small number of samples, or it may also indicate that tryptophan level is a more significant marker. In addition, it seems that the method of measuring IDO activity may be a possible reason for no difference between the groups in Kyn/Trp ratio.

Thyroid diseases are considered to be associated with oxidative stress. In particular, it has long been known that there may be a relation between PTC and autoimmunity.[40] Thyrocytes often produce reactive oxygen species (ROS) in low amounts which are physiologically necessary for thyroid hormone synthesis. Thyroid gland is an organ where oxidative reactions are active due to secreted hormones.[18] Many defense systems such as peroxiredoxins, CAT and GPx are active against ROS formation to protect cell integrity in thyrocytes. However, excessive ROS production can cause toxicity in thyrocytes, and the disturbances in this pathway may have a role in autoimmune thyroid disorders.[41,42] Increased SOD and CAT activities are observed in malignant thyroid diseases. High SOD activity in thyroid diseases is a defense mechanism against excessive production of oxygen radicals. On the other hand, low CAT activity can be observed due to depletion of CAT enzyme secondary to increased oxidative stress and hydrogen peroxide. The CAT activity is generally reduced at the initial stage of excessive oxygen radical production. In our study, we found lower CAT activities due to increased oxidative stress in thyroid diseases.

The oxidant-antioxidant balance is disturbed in thyroid diseases. In one study, it was found that CAT and SOD enzyme activities in tumor tissue of head and neck epidermoid cancers gradually decreased as the stage advanced.[43] In another study, while SOD activity in patients with malignant tumors in head and neck region was higher than control group, CAT activity decreased.[44] In the study by Durak et al., it was reported that SOD activity in thyroid cancer tissues was lower than tissues without thyroid cancer, and there was no difference in CAT activity between tissues. It was shown that the decrease in SOD activity may be spontaneous due to the cancer process, and that no change in CAT activity may be due to peroxisomal localization in cancerous thyroid tissues. Low enzyme activities occur as a result of alteration of cellular homeostasis in thyroid cancer tissues.[45] In a study on antioxidant enzymes in different thyroid diseases, it was suggested that the level of antioxidant enzymes increased to suppress increased ROS production.[40]

Serum neopterin levels are elevated when cellular immune system is activated. Increased neopterin levels are observed in cancer patients due to immune system activation. In addition, the measurement of neopterin levels at the time of recurrence may give an idea about the stage of the disease.[46] In our study, we found increased neopterin levels in patients with PTC compared to patients with benign thyroid diseases. Also, the results of a study by Beksac et al were similar to our study. They found higher neopterin levels in patients with PTC and suggested that the changes in neopterin concentrations might give an idea about the type of cancer.[25] The most important values of the present study are the differences in tryptophan and neopterin levels between malignant and benign patients groups. Based on these results, we showed that neopterin and tryptophan levels might be used as tumor markers in highly suspected cases to distinguish malignant tumors from benign tumors. We think that these two biomarkers can play an important role in early diagnosis of PTC.

When the correlations between parameters were considered, a significant positive correlation was found between kynurenine levels and IDO activation in all study groups including control group. A negative correlation was observed between tryptophan levels and IDO activation. Likewise, a negative correlation was also found between SOD and CAT enzyme activities.

In our study, we investigated the relationship between immune system parameters and antioxidant enzyme activity in benign and malignant thyroid diseases. We think that further studies are needed to support our results. We suggest that kynurenine and tryptophan levels could be supportive indicators for diagnosis of thyroid diseases as well as CAT activity for monitoring diseases. In the guidance of the current results, we started another analysis of these parameters at the tissue level in benign and malignant thyroid diseases.

Authors' Contribution

TB conceived the study. SÜ, GG, TTŞ, BK, FT, and OY supervised the conduct of the study and data collection. SÜ, and GG provided statistical advice on study design and analyzed the data. SÜ drafted the manuscript and all authors contributed substantially to its revision.

Conflict of Interest Disclosures

The authors have no conflicts of interest.

Ethical Statement

The present study was approved by the local Ethics Committee of the Medical Faculty of Gazi University to be in concordance with the Helsinki Declaration, 1981. Each patient recruited to the study was given an information guide and asked for informed consent.

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