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The evaluation of dynamic thiol/disulfide homeostasis in papillary thyroid carcinoma

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Abstract

Aim: The aim of this presented article is to analyze the correlation between thiol/disulfide homeostasis (TDH) and papillary thyroid carcinoma (PTCA) and to investigate whether there is an effect of TDH on the development of PTCA.

Materials and Methods: Preoperative venous blood sampling was obtained from the patients having applied to our hospital due to euthyroid nodular goiter and detected with suspected malignancy or malignant cytology in FNAB, according to the BETHESDA system. Those whose postoperative histopathological results proved the presence of PTCA were involved in this study. Healthy volunteers who were without any thyroid nodules and thyroid dysfunction.

Results: The number of participants in the patient and control groups was 34 and 101, respectively. Compared to the control group, native thiol (µmol/L), total thiol (µmol/L), and native thiol/total thiol × 100 values were ascertained to be lower in the patient group with PTCA; however, these values were not statistically significant. Disulfide, disulfide/native thiol × 100, and disulfide/total thiol × 100 values were detected to be lower in the patient group with PTCA, but not statistically significant. When the patients with PTCA were assessed within themselves, TDH parameters were not associated with the PTCA subtype, the number of foci, the presence of lymphovascular invasion, and the presence of metastatic lap.

Conclusion: Considering the parameters of PTCA and TDH, the results were not statistically significant. And besides postoperative histopathological findings such as the PTCA subtype, the number of foci, the presence of lymphovascular invasion, and presence of metastatic lap were not correlated with THD parameters.

Introduction

To demonstrate the presence of the nodule, thyroid ultrasound ought to be performed in all patients with suspicious nodules [1]. Thyroid nodules are frequently encountered today owing to the increasing prevalence of ultrasonography. The clinical significance of thyroid nodules derives from the probability of contracting thyroid cancer, which is 4-6.5% [2]. The preferred method for the evaluation of thyroid nodules is fine-needle aspiration biopsy (FNAB) [1]. The cytological specimen obtained by biopsy is generally evaluated in accordance with the BETHESDA system. According to this system, there are six categories: non-diagnostic, benign, atypia of undetermined significance/follicular lesions of undetermined significance (AUS/FLUS), follicular neoplasm (FN) or suspected FN, suspicion of malignancy, and malignant [3]. Surgery is usually recommended if the FNAB result is suspected or malignant [1].

Thiols are organic compounds, containing a sulfhydryl (-SH) group formed by the bonding of sulfur (S) and hydrogen (H) atoms to the carbon atom [4, 5]. Also, they constitute a large part of antioxidants and provide defense against reactive oxygen species (ROS) via their role in redox homeostasis [5]. Functional sulfhydryl (-SH) groups in thiols act as substrates and free radical scavengers for an-

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tioxidant enzymes. The majority of the plasma thiol pool is made up of albumin and other plasma proteins, whereas a small proportion consists of low-molecular-weight thiols such as cysteine, cysteinyl glycine, glutathione, homocysteine, and γ-glutamylcysteine [6]. Thiol groups convert to their reversible forms called disulfide bonds (–S–S–) under oxidative stress. These bonds may be come down back to the thiol groups, thereby maintaining dynamic thiol-disulfide homeostasis (TDH) [5]. That abnormal thiol disulfide homeostasis plays a significant role in the pathogenesis of several cancer types has been proven [7–9].

Only one side of this bilateral balance has been measured since 1979. However, thanks to the new method developed by Erel & Neşelioğlu, both variable levels may be measured separately and cumulatively, and they also may be evaluated both individually and holistically [5].

The primary aim of this study is to analyze the correlation between thiol/disulfide homeostasis (TDH) and papillary thyroid carcinoma (PTCA) and to reveal the possible impact of TDH on the development of PTCA.

Materials and Methods

Our study is a case-control study. Preoperative venous blood sampling was obtained from the patients having applied to Diskapi Yildirim Beyazit Training and Research Hospital, Clinic of Endocrinology and Metabolic Diseases between 2019 and 2020 due to euthyroid nodular goiter and detected with suspected malignancy or malignant cytology in fine-needle aspiration biopsy, according to the BETHESDA system. The thyroid surgery multidisciplinary council being held every week in our center decided to perform thyroidectomy, and the patients referred for the operation. Indications for thyroidectomy were the reporting of suspected malignancy or malignant cytology in fine-needle aspiration biopsy. Those whose postoperative histopathological results proved the presence of PTCA were involved in this research; however, the exclusion criteria were as below: using cigarettes and/or alcohol, having chronic diseases such as diabetes mellitus, cardiovascular and cerebrovascular diseases, kidney and liver diseases, rheumatic diseases, and malignancy, and drug use (lipid-lowering agents, etc.). Age- and gender-matched healthy volunteers without any thyroid nodules and thyroid dysfunction and those meeting the exclusion criteria constituted the control group. Venous blood sampling was obtained from the two groups following 12-hour fasting. To distinguish serum and plasma, blood samples were centrifuged at 1500 rpm for 10 minutes. Serum samples were stored at minus 80 degrees until the end of the study. Preoperative thyroid functions like serum thyrotropin (TSH), free triiodothyronine (fT3), free thyroxine (fT4), and cytology and histopathology results were obtained from medical records.

Ultrasonography was performed by experienced endocrinologists via color Doppler ultrasonic diagnostic device (Hitachi, Japan; EUB 7000-13-MHz linear probe).

Native thiol and total thiol levels as well as disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol levels were measured via a latest and fully automated system [5].

The Declaration of Helsinki was based on over the course of the study approved by University of Health Science Diskapi Yildirim Beyazit Training and Research Hospital Ethics Committee (Ref. No: 72/09). Written informed consent of all participants were also obtained.

Statistical analysis

In order to determine if the variables were normally distributed, visual (probability plots and histograms) and analytic (Kolmogorov-Smirnov/Shapiro-Wilk's test) methods were used. Disulfide, native thiol, and total thiol levels, disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol levels ratios were compared in terms of age factor via the Student's t-test. Categorical data were presented as numbers and percentages (%). The methods used in order to show descriptive analysis were means and standard deviation for normally distributed variables and medians and min-max for non-normally distributed ones. A p- value below 0.05 was accepted statistically significant. Correlations was determined through Spearman's correlation analysis.

Results

The number of the participants in the patient and control groups was 34 and 101, respectively. The mean age was 53.7 ± 12.2 and 47.8 ± 9.1 years in the patient group with PTCA and in the control group, respectively (p=0.013). Twenty-seven (79.4%) patients with PTCA and 73 (72.3%) of the control group were female (p=0.412). The TSH value was 1.67 µIU/mL (0.37-6.1) in the patient group with PTCA, whereas 1.67 µIU/mL (0.45-4.8) in the control group (p=0.728). On the other hand, the fT4 value was 1.01 (ng/dL) (0.69-1.7) in the patient group with PTCA, while 0.81 ng/dL (0.63-1.44) in the control group (<0.001).

The thyroid tissue was reported as homogeneous in 4 (11.8%), slightly heterogeneous in 25 (73.5%), and moderately heterogeneous in 5 (14.7%) patients in the preoperative ultrasonography of the patients with PTCA. The mean number of nodules was 3.5 (1-10), and the mean diameter of the nodule considered to be malignant was

Table 1. Preoperative characteristics of patients with PTCA.

Parenchymal Heterogeneity n, %	
Normal	4 (11.8)
Mild	25 (73.5)
Moderate	5 (14.7)
Advanced	0 (0)
Number of nodules n, %	3.5 (1-10)
Malignant nodule largest diameter, mm	14.5 (4-70)
Ultrasonographic lap presence n, %	8 (23.5)
Ultrasonographic lap number n,	% 2 (1-7)
Ultrasonographic lap largest diameter, mm	17 (5-29)
Preoperative FNAB result	
Suspicion of malignancy n, %	24 (70.6)
Malignant n, %	10 (29.4)

LAP: Lymphadenopathy, FNAB: Fine-needle Aspiration Biopsy.

Table 2. Postoperative characteristics of patients with PTCA.

Papillary thyroid carcinoma subtypes n, %	
Classical	19 (55.9)
Follicular	13 (38.2)
Hobnail	1 (2.9)
Solid	1 (2.9)
Focality n, %	
Unifocal	19 (55.9)
Multifocal	15 (44.1)
Number of foci n, %	2 (2-10)
Largest focus, mm	11 (2-65)
Capsular invasion n, %	10 (29.4)
Vascular invasion n, %	5 (14.7)
Extracapsular spread n, %	4 (11.8)
Operation n, %	
Lobectomy	2 (5.9)
Total thyroidectomy	17 (50)
Total thyroidectomy + unilateral SLND	6 (17.6)
Total thyroidectomy + bilateral SLND	4 (11.8)
Total thyroidectomy + bilateral SLND + lateral	3 (8.8)
Total +bilateral +bilateral lateral	2(5.9)
Metastatic LAP presence	7 (20.6)
Metastatic LAP number	4 (2-17)

LAP: Lymphadenopathy.

Table 3. Comparison of biochemical measurements between thyroid papillary cancer patients and control group.

	Thyroid papillary cancer (n=34)	Control group (n=101)	P value
Disulfide (µmol/L)	18.3 ± 3.5	17.5 ± 5.1	0.302
Native thiol (µmol/L)	492.4 ± 71.6	508.5 ± 47.7	0.235
Total thiol (µmol/L)	529 ± 73.8	543.4 ± 49.6	0.302
Disulfide/native thiol × 100	3.8 ± 0.8	3.45 ± 1.04	0.073
Disulfide/total thiol × 100	3.5 ± 0.7	3.2 ± 0.9	0.065
Native thiol/total thiol × 100	93 ± 1.4	93.6 ± 1.8	0.065

14.5 mm (4-70). When examining the results of the preoperative fine-needle aspiration biopsy, 24 (70.6%) were reported as suspected malignancy and 10 (29.4%) as malignant cytology. Other preoperative ultrasonographic features of the patients and the operations performed are presented in Table 1.

Considering the postoperative pathology results of the patients with PTCA, 19 (55.9%) patients had a classical type, 13 (38.2%) follicular type, 1 (2.9%) hobnail subtype, and 1 (2.9%) solid subtype. Moreover, the number of patients reported as unifocal and multifocal was 19 (55.9%) and 15 (44.1%), respectively. The average number of foci was 2 (2-10). The mean size of the largest focus was 11 mm (2-65). Other data regarding postoperative pathological evaluation are summarized in Table 2.

Compared to the control group, native thiol (µmol/L), total thiol (µmol/L), and native thiol/total thiol × 100 values were found to be lower in the patient group with PTCA; however, these values were not statistically significant (p=0.235, p=0.302, p=0.065, respectively) (Table 3). On the contrary, disulfide, disulfide/native thiol × 100, and disulfide/total thiol × 100 values were found to be higher in the patient group with PTCA, not statistically significant (p=0.302, p=0.073, p=0.065, respectively).

When the patients with PTCA were assessed within themselves, TDH parameters were not associated with the PTCA subtype, the number of foci, the presence of lymphovascular invasion, and the presence of metastatic lap.

Discussion

Given our study results, compared to the control group, native thiol (µmol/L), total thiol (µmol/L), and native thiol/total thiol × 100 values were lower in the patient group with PTCA. However, disulfide, disulfide/native thiol × 100, and disulfide/total thiol × 100 values were higher. This increase was not associated with the PTCA subtype, the number of foci, the presence of lymphovascular invasion, and the presence of metastatic lap. Organic compounds containing the sulfhydryl group are called thiol (-SH), which consists of sulfur and hydrogen atoms. Thiols are highly susceptible to oxidation due to -SH groups. Disulfides (-S-S-) are the most significant type of dynamic, redox-reactive covalent bonds that were formed between two thiol groups. Dynamic TDH is the reversal of thiol oxidation in proteins, and it represents the levels of thiols and disulfides. It is a key parameter associated with many biochemical processes, including regulation of protein function, stabilization of protein structure, protection of proteins against irreversible oxidation of cysteine residues, chaperone function, regulation of enzyme functions, and transcription [10]. Although TDH has been studied previously in several types of cancer, the results remain inconsistent. While thiol levels were found to be lower in patients with breast cancer, disulfide values were found to be higher [7]. The plasma disulfide levels in cervical cancer patients were higher than the control group whereas native thiol and total thiol levels were lower. Nevertheless, no statistically significant difference was observed. There was a statistically significant positive correlation between disulfide level and the stage of cervical cancer [9]. The mean plasma level of native thiol and total thiol was lower in the patients with prostate cancer, while plasma disulfide levels were not statistically different between the groups [11].

Considering the studies on thyroid cancer, the levels of native thiol, total thiol, and disulfide levels were detected to be lower in patients with thyroid cancer than in controls in a study conducted with 23 patients and 20 controls. On the other hand, disulfide/native thiol and disulfide/total thiol levels were detected to be higher in patients with thyroid cancer; however, this increase was not statistically significant, similar to our study [12].

In another study conducted by Bilginer et al. in Türkiye, contrary to our study, the disulfide/native thiol and the disulfide/total thiol levels were significantly higher in postoperative histopathologically malignant patients compared to benign ones. No difference was observed in these values between the control group and the patient group with benign. According to the results of the study, there was a significant positive correlation between the increased risk of malignancy with regard to preoperative cytology and disulfide, disulfide/native thiol, and disulfide/total thiol levels [13].

The relationship between THD parameters and PTCA has not been clearly observed in previous studies. The conflicting data make it difficult to understand how important oxidative stress is in the development of PTCA. Therefore, larger studies are needed.

Considering the content of our study, to the best of our knowledge, there is no study in the literature examining the association between the thiol/disulfide homeostasis and postoperative histopathological parameters. The fact that the population size is limited and that the parameters may be affected by many variables are the shortcomings of our study.

We could not detect a significant association between the PTCA and THD parameters. There was no correlation between postoperative histopathological findings and THD parameters. Nevertheless, further studies are required to reveal the role of TDH parameters on the diagnosis and treatment of PTCA.

Ethical approval

The research was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the medical ethical commission of University of Health Science Diskapi Yildirim Beyazit Training and Research Hospital (reference: 72/09). Informed consent was obtained from all participants included in the study.

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