

Research Article

Can dynamic thiol-disulfide homeostasis be an effective marker in the diagnosis of nodular goiter and thyroid cancer?

Dinamik tiyol-disülfür homeostazisi nodüler guatr ve tiroid kanseri tanısında etkili bir belirteç olabilir mi?

 Altan Aydın*¹,  Hakan Bulus²,  Murat Alisik³,  Ozcan Erel⁴

¹Department of General Surgery, Health Sciences University, Kanuni Education and Research Hospital, Trabzon, Turkey,

²Department of General Surgery, Health Sciences University, Kecioren Education and Research Hospital, Ankara, Turkey,

³Department of Biochemistry, Abant İzzet Baysal University Hospital, Bolu, Turkey,

⁴Department of Biochemistry, Yildirim Beyazıt University Hospital, Ankara, Turkey.

Abstract

Aim: The oxidative stress has an important role in thyroid pathologies by nature of thyroid gland. Dynamic thiol-disulfide homeostasis is one of the markers of oxidative stress and its counterpart antioxidants in the body. In our study, the dynamic thiol-disulfide homeostasis was investigated in cases underwent surgery due to thyroid cancer or nodular goiter.

Material and Methods: The study included patients who underwent thyroidectomy in General Surgery Department of Keçiören Teaching and Research Hospital between 01.03.2017 and 01.06.2017. The patients were assigned into groups according to postoperative histopathological examination: group 1 included patients with benign lesion in histopathology report and group 2 included patients with malignant lesions in histopathology report. The patients who had no pathology in sonography and did not undergo surgery were assigned into group 3 as controls. In all patients, venous blood samples were drawn to evaluate dynamic thiol-disulfide homeostasis before surgery.

Results: In the study, 98 cases underwent bilateral total thyroidectomy; 77 of which had benign disease and 21 of which had malignant disease. Native thiol values ($\mu\text{mol/L}$) were 317.4 ± 4.2 , 349.9 ± 7.9 and 299.9 ± 7.9 ($p=0$) while total thiol values ($\mu\text{mol/L}$) were 353.5 ± 4.8 , 386.5 ± 9.5 and 332.6 ± 8.3 ($p=0$) and disulfide values ($\mu\text{mol/L}$) were 18.4 ± 0.5 , 20.5 ± 0.7 and 16.7 ± 0.6 ($p=0$) in group 1 (benign disease), group 2 (malignant disease) and group 3 (controls), respectively. In addition disulfide: native thiol was 5.8 ± 0.1 , 5.9 ± 0.2 and 5.7 ± 0.2 ($p=0.8$) while disulfide: total thiol was 5.2 ± 0.1 , 5.4 ± 0.2 and 5.1 ± 0.2 and native thiol: total thiol was 89.9 ± 0.5 , 90.7 ± 0.5 and 90.5 ± 1.5 ($p=0.4$) in group 1, 2 and 3, respectively.

Conclusion: The dynamic thiol-disulfide homeostasis can be used as a marked in the thyroid diseases; however, further studies with larger sample are needed.

Keywords: goiter, thiol-disulfide, thyroid diseases

Corresponding Author*: Altan Aydın, Department of General Surgery, Health Sciences University, Kanuni Education and Research Hospital, Trabzon, Turkey.

Orcid: 0000 0002 2981 2833

E-mail: altanaydin76@hotmail.com

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Öz

Amaç: Troid glandı doğası gereği troid patolojilerinde oksidatif stresin rolü önemlidir. Dinamik tiol-disulfid dengesi vücuttaki oksidatif stres ve onun dengeleyicisi antioksidanların göstergelerinden birisidir. Çalışmamızda Troid kanseri veya nodüller guatr nedeniyle opere edilen olgularda Dinamik tiol disulfid dengesi düzeylerini araştırdık.

Gereç ve Yöntemler: Keçiören Eğitim ve Araştırma hastanesi Genel Cerrahi kliniğinde 01.03.2017-01.06.2017 tarihleri arasında tiroidektomi operasyonu olan hastalar çalışmaya dahil edildi. Ameliyat sonrası patoloji sonuçları Benign gelenler Grup1, Malign gelenler Grup 2 ve Ultrasonda patoloji saptanmayan ve ameliyat edilmeyen olgular ise Grup 3 Kontrol grubuna dahil edildi. Çalışma grubundan ameliyat öncesi olmak üzere Dinamik tiol-disulfid dengesinin araştırılması için venöz kan örnekleri alındı.

Bulgular: 98 hastaya bilateral total tiroidektomi ameliyatı uygulandı; bunlardan, 77 hastanın patoloji sonuçları benign, 21 hastanın ise malign rapor edildi. Grup 1 (benign), Grup 2 (malign) ve Grup 3 (kontrol) gruplarında sırasıyla Native tiol değerleri ($\mu\text{mol/L}$) 317.4 ± 4.2 , 349.9 ± 7.9 , 299.9 ± 7.9 ($p=0$), Total Thiol değerleri ($\mu\text{mol/L}$); $353.5.0 \pm 4.8$, 386.5 ± 9.5 , 332.6 ± 8.3 ($p=0$), Disulfid ($\mu\text{mol/L}$); 18.4 ± 0.5 , 20.5 ± 0.7 , 16.7 ± 0.6 ($p=0$), Disulphide/native tiol $5.8.0 \pm 0.1$, 5.9 ± 0.2 , 5.7 ± 0.2 ($p=0.8$), Disulphide/total tiol 5.2 ± 0.1 , 5.4 ± 0.2 , 5.1 ± 0.2 ($p=0.7$) ve Native tiol/total 89.9 ± 0.5 , 90.7 ± 0.5 , 90.5 ± 1.5 ($p=0.4$) olarak saptandı.

Sonuç: Tiroid hastalıkları tanısında Dinamik tiol-disulfid dengesi bir belirteç olarak kullanılabilir ancak geniş serili çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: guatr, tiyol-disülfür, tiroid hastalıkları

Introduction

Oxidative balance is important in the thyroid gland due to oxidative mechanisms involved in thyroid hormone synthesis; and thyroid gland is highly vulnerable against oxidative injury. The thyrocytes produces reactive oxygen metabolites (namely, hydrogen peroxide) which are important in the final step of thyroid hormone synthesis. The excessive or insufficient production of these metabolites may lead thyroid gland injury as a result of DNA strand breaks, mutations or apoptosis. Thus, it has been proposed that oxidative stress may be involved in many disorders in the thyroid gland including thyroid nodules, thyroid cancer or autoimmune thyroiditis [1-4].

In human body, there are several mechanisms to prevent free radical formation and their harmful effects. These mechanisms are generally termed as antioxidants. The antioxidants provide protection against cell damage by preventing free radical formation. There are several thiol compound containing sulfhydryl groups such as glutathione (GSH), cysteine and N-acetylcysteine. Thiols may form disulfide (RSSR) bound against oxidants by oxidation reaction. Under oxidative stress, oxidation of cysteine residues may lead mix disulfide structures between protein thiol groups and low-molecular mass thiols. The disulfides formed may be re-reduced in to thiol groups;

thus, dynamic thiol-disulfide homeostasis can be maintained.

The dynamic thiol-disulfide homeostasis is one of the markers for oxidative stress which is implied in the pathogenesis of thyroid nodules and thyroid cancers and its counterpart antioxidants [4]. In this study, we intended to investigate role of dynamic thiol-disulfide homeostasis in the pathogenesis of nodular goiter and thyroid cancer.

Material and Methods

The study was approved by Ethics Committee on Clinical Research of Keçiören Teaching and Research Hospital. The study included patient who underwent surgery due to giant goiter, malignant cytology, follicular neoplasm, AUS/FUS and with cosmetic indication at General Surgery Department of Keçiören Teaching and Research Hospital between 01.03.2017 and 01.06.2017. The patients with hyperthyroidism, hypothyroidism, cardiovascular disease, diabetes mellitus chronic hepatic or renal disease and those with history of smoking and alcohol consumption were excluded. The patients who had no nodule on thyroid sonography and normal thyroid functions tests were included as controls.

In the study and control groups, the venous blood samples were drawn into EDTA tubes following 12-hours fasting in order to assess thiol-disulfide homeostasis. Plasma was obtained by

centrifugation at 1500 rpm over 10 minutes. Plasma samples were stored at -80°C until assays. During thiol-disulfide homeostasis tests, disulfide bounds were initially reduced to form free functional thiol groups. Formaldehyde was used to remove sodium borohydride unused and consumed. Total thiol (-SF+-S-S) and native thiol (-SH) levels were quantified using Ellman's and modified Ellman's reagents. The amount of native thiol was subtracted from total thiol; the difference was divided by two, indicating amount of dynamic disulfide bounds (-S-S). Using these parameters, ratios for disulfide: native thiol $[(-S-S) \times 100 / (-SH)]$, disulfide total thiol $[(-S-S) \times 100 / (-SH + -S-S)]$ and native thiol: total thiol $[(-SH) \times 100 / (-SH + -S-S)]$ were calculated.

In the study groups, total thyroidectomy or lobectomy with isthmusectomy were performed based on the diagnosis. The patients were assigned into groups according to postoperative histopathological examination: group 1 included patients with benign lesion in histopathology report and group 2 included patients with malignant lesions in histopathology report. The patient who had no pathology in sonography and did not undergo surgery were assigned into group 3 as controls.

Statistical analyses were performed using SPSS version 22. Chi-square test was used to compare groups regarding gender. The normal distribution of variables across benign and malignant groups were assessed using visual plots and analytic methods. Variables with normal distribution were compared using Student's t test. Variables with skewed distribution were compared using Mann Whitney U test. The normal distribution of variables across benign, malignant and control groups were assessed using visual plots and analytic methods. Variables with normal distribution were compared using Kruskal-Wallis test across groups. Variables

with skewed distribution were compared using one-way ANOVA. The homogeneity of variance was assessed using Levene's test. In case of statistical significant across groups, binary comparisons were performed using post-hoc Tukey test. A p value < 0.05 was considered as statistically significant.

Results

In the study groups underwent surgery due to thyroid gland pathology, there were 60 women (77.9%) and 17 men (22.1%) in the group 1 (benign disease) whereas 19 women (90.4%) and 2 men (9.6%) in the group 2 (malignant disease). In the control group, there were 34 women (68.0%) and 16 men (32.0%). Mean age was 51.2 ± 1.5 years in the group 1, 48.0 ± 3.1 years in the group 2 and 39.7 ± 2.2 years in the group 3. There was no significant difference in gender distribution across groups. Among patients underwent surgery, histopathological examination reported as benign disease (nodular hyperplasia, benign colloid nodule, lymphocytic thyroiditis, Hashimoto thyroiditis) in 77 (78.6%) and malignant disease in 21 (21.4%). Of 21 patients with malignant pathology, 19 (90.5%) had papillary carcinoma while 2 (9.5%) had follicular carcinoma (Table 1).

Native thiol values ($\mu\text{mol/L}$) were 317.4 ± 4.2 , 349.9 ± 7.9 and 299.9 ± 7.9 ($p=0$) while total thiol values ($\mu\text{mol/L}$) were 353.5 ± 4.8 , 386.5 ± 9.5 and 332.6 ± 8.3 ($p=0$) and disulfide values ($\mu\text{mol/L}$) were 18.4 ± 0.5 , 20.5 ± 0.7 and 16.7 ± 0.6 ($p=0$) in group 1 (benign disease), group 2 (malignant disease) and group 3 (controls), respectively. In addition disulfide: native thiol was 5.8 ± 0.1 , 5.9 ± 0.2 and 5.7 ± 0.2 ($p=0.8$) while disulfide: total thiol was 5.2 ± 0.1 , 5.4 ± 0.2 and 5.1 ± 0.2 and native thiol: total thiol was 89.9 ± 0.5 , 90.7 ± 0.5 and 90.5 ± 1.5 ($p=0.4$) in group 1, 2 and 3, respectively.

Table 2. Demographic characteristics of the groups

		Group 1 (Benign) (n:77)	Group 2 (Malignant) (n:21)	Group 3 (Control) (n:50)	P value
Age		51.2±1.5	48.0±3.1	39.7±2.2	0.0*
Sex	Female	60 (77.9%)	19 (90.4%)	34 (68%)	0.1**
	Male	17 (22.1%)	2 (9.6%)	16 (32%)	
		Nodular hyperplasia n:32 (41.6%)	Papillary carcinoma n:19 (90.5%)		
		Benign colloid nodule n:34 (44.2%)	Follicular carcinoma n:2 (9.5%)		
		Follicular adenoma n:4 (5.2%)			
		Lymphocytic thyroiditis n:5 (6.5%)			
		Hashimoto's thyroiditis n:2 (2.6%)			

* One-way ANNOVA test
** Chi-square test

Discussion

Reactive oxygen species and free radicals play role in many metabolic processes in the human body. There is a physiological balance between ROS production and detoxification. The disruption of the balance due to internal or external factors results in oxidative stress, playing major role in the pathogenesis of many disorders. Hydrogen peroxide (H₂O₂) generated by NADPH oxidase is a form of ROS, which is ubiquitously produced by every single cell in the body at varying amounts. The amounts of H₂O₂ produced above physiological levels leads oxidative stress, resulting in DNA damage and mutations. Thyroid gland has an oxidative nature as substantial amount of ROS, particularly H₂O₂, is essential for thyroid hormone synthesis. It has been shown that antioxidant enzymes such as superoxide dismutase (SOD), glutathione (GSH), peroxidase (GSH-Px) and catalase as well as α- and γ-tocopherols, Co-enzyme Q and ascorbic acid have a role in the thyroid gland. Among antioxidants, peroxiredoxins (Prxs) have an unique value due its role in H₂O₂ elimination and prevention of H₂O₂-associated apoptosis [2, 6]. In our study, we aimed to investigate the oxidative stress and antioxidant homeostasis in the thyroid glands where oxidative stress is intense, and to clarify their role in the disease pathogenesis.

Thiol compounds containing sulfhydryl groups are involved in the thiol-disulfide homeostasis, including glutathione (GSH), cysteine or N-acetylcysteine with antioxidant effects. Thiols can form disulfide bounds (RSSR) by oxidation reaction against oxidants. Under oxidative stress, oxidation of cysteine residues may lead mix disulfide structures between protein

thiol groups and low-molecular mass thiols. The disulfides formed may be re-reduced in to thiol groups; thus, dynamic thiol-disulfide homeostasis can be maintained [7]. In our study, we evaluated oxidative balance underlying thyroid pathologies through thiol-disulfide homeostasis.

Given that goiter is frequently seen with familial pattern and compatible with autosomal dominant inheritance, the genetic factors as well as environmental factors are commonly proposed as underlying mechanisms. The nodule formation is triggered by oxidative stress due to oxidative nature of thyroid hormone synthesis or induced due to factors such as iodine deficiency or smoking. If antioxidant defense system fails, this may cause nodule formation by leading DNA damage and mutations [8]. In our study, disulfide and thiol levels were significantly higher in patients with nodular goiter when compared to controls. The higher levels of disulfide compared to controls showed the role of oxidative stress in nodular goiter. However, thiol levels were also significantly higher when compared to controls. In agreement with our study, Bilginer et al. reported higher thiol levels, albeit insignificant, in benign thyroid diseases when compared to control group [13].

In cancer, redox equilibrium is also impaired; preventing antioxidant and detoxification proteins to counter oxidative stress by increasing intra- and extra-cellular ROS levels. The oxidative stress triggered leads development of malignant phenotype by inducing several processes such as angiogenesis, proliferation, invasion and apoptosis [2, 3, 9]. In the studies on oxidative state in thyroid cancers, high levels of oxidants were detected; however, antioxidant levels might vary. The

SOD activity was found to be lower when compared to normal tissues in the studies by Sugawara et al. and Durak et al. while Akinci et al. observed a slight decrease in preoperative SOD values after surgery in patients underwent thyroidectomy [10-12]. In our study, disulfide levels as well as thiol values were found to be significantly higher when compared to controls.

In the study by Bilginer et al., 81 cases underwent surgery due to thyroid pathology and they were assigned into benign and malignant disease groups after surgery. The study groups were compared with controls. It was found that native thiol ($\mu\text{mol/L}$) was found to be 457.47 ± 62.38 , 453.19 ± 63.49 and 444.81 ± 71.9 ($p=0.477$) while total Thiol ($\mu\text{mol/L}$) was 497.09 ± 64.78 , 487.45 ± 67.87 , and 474.61 ± 75.48 , and disulfide ($\mu\text{mol/L}$) was 19.85 ± 11.28 , 16.07 ± 9.28 , $14.87 \pm$ and 7.62 0.191 across groups. Although total thiol, native thiol and disulfide levels were found to be higher in malignant disease group when compared to benign disease and control groups, the difference did not reach statistical significance (13). In our study, native thiol values ($\mu\text{mol/L}$) were 349.9 ± 7.9 , 317.4 ± 4.2 and 299.9 ± 7.9 ($p=0$) while total Thiol values ($\mu\text{mol/L}$) were 386.5 ± 9.5 , 353.5 ± 4.8 , and 332.6 ± 8.3 ($p=0$) and disulfide values ($\mu\text{mol/L}$) were 18.4 ± 0.5 , 20.5 ± 0.7 , 16.7 ± 0.6 ($p=0$) in group 1, 2 and 3, respectively. In agreement with the study by Bilginer et al., total thiol, native thiol and disulfide values were found to be significantly higher in malignant group when compared to benign and control groups. This can be explained by oxidative nature of thyroid gland. Given the oxidative nature, it may result in higher antioxidant release even under oxidative stress.

In our study, there were 21 patients with thyroid cancer. Due to limited number of cases, it is difficult to draw conclusions about use thiol-disulfide homeostasis as a marker in the thyroid cancer. In addition, the mechanisms underlying thyroid pathologies are different; however, we classified these pathologies in two major groups as benign and malignant. We think that it will more appropriate to investigate thyroid pathologies separately, requiring studies with larger sample size. However, our study is valuable in clarifying role of thiol-disulfide homeostasis in thyroid pathologies.

Conclusion

Oxidative stress has an important role in the thyroid pathologies due to nature of thyroid gland. Oxidants are involved in thyroid cancer process. Thiol-disulfide homeostasis, a marker of oxidative state, can be a marker in thyroid pathologies. Further studies with larger sample size are needed in this issue.

References

1. Paschke R, Hegedüs L, Alexander E, Valcavi R, Papini E, Gharib H. Thyroid nodule guidelines: agreement, disagreement and need for future research. *Nat Rev Endocrinol.* 2011 Jun;7(6):354-61. Doi: 10.1038/nrendo.2011.1. Epub 2011 Mar
2. Kochman J, Jakubczyk K, Bargiel P, Janda-Milczarek K. The Influence of Oxidative Stress on Thyroid Diseases. *Antioxidants (Basel).* 2021 Sep 10;10(9):1442. doi: 10.3390/antiox10091442.
3. El Hassani, R.A.; Buffet, C.; Leboulleux, S.; Dupuy, C. Oxidative stress in thyroid carcinomas: Biological and clinical significance. *Endocr.-Relat. Cancer* 2019, 26, R131–R143.
4. Alkurt E. G., Turhan S., Şahin F., Tutan M. B. Papiller Tiroid Kanseri Hastalarda Preoperatif Santral Lenf Nodu Metastazının Öngörücüsü Olarak Glikoz-Lenfosit Oranı. *Turkish Journal of Clinics and Laboratory.* 2022; 13(4): 534-539.
5. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clinical Biochemistry.* 2014;47(18):326–332. doi: 10.1016/j.clinbiochem.2014.09.026.
6. Ünsal, A., Yavuz, A., Buluş, H., Aydın, A., Alışık, M., Erel, Ö., & Aydın Sr, A. (2022). Comparison of Thiol-Disulfide Hemostasis With Inflammatory Markers in the Inflammatory Process of Acute Appendicitis. *Cureus*, 14(9).
7. Song, Y.; Driessens, N.; Costa, M.; De Deken, X.; Detours, V.; Corvilain, B.; Maenhaut, C.; Miot, F.; Van Sande, J.; Many, M.-C.; et al. Roles of Hydrogen Peroxide in Thyroid Physiology and Disease. *J. Clin. Endocrinol. Metab.* 2007
8. Paschke, R. Molecular pathogenesis of nodular goiter. *Langenbecks Arch. Surg.* 396, 1127–1136 (2011).
9. Muzza, M.; Pogliaghi, G.; Colombo, C.; Carbone, E.; Cirello, V.; Palazzo, S.; Frattini, F.; Gentilini, D.; Gazzano, G.; Persani, L.; et al. Oxidative Stress Correlates with More Aggressive Features in Thyroid Cancer. *Cancers* 2022, 14, 5857
10. Sugawara M, Kita T, Lee ED, Takamatsu J, Hagen GA, Kuma K, Medeiros-Neto GA. Deficiency of superoxide dismutase in endemic goiter tissue. *J Clin Endocr Metab.* 1988;67:1156-61.
11. Durak I, Bayram F, Kavutcu M, Canbolat O, Öztürk HS. Impaired enzymatic antioxidant defence mechanism in cancerous human thyroid tissues. *J Endocrinol Invest.* 1996;19:312-5
12. Akinci, M.; Kosova, F.; Çetin, B.; Sepici, A.; Altan, N.; Aslan, S.; Cetin, A.; Çetin, B. Oxidant/antioxidant balance in patients with thyroid cancer. *Acta Cir. Bras.* 2008, 23, 551–554.
13. Bilginer MC, Tam AA, Fakı S, Güler BY, Erel Ö, Kılınç İ, Özdemir D, Topaloğlu O, Ersoy R, Çakır B. Association of thiol/disulphide homeostasis with Bethesda classification of thyroid nodules and thyroid cancer. *Turk J Med Sci.* 2022; 52(4): 990–996. doi: 10.55730/1300-0144.5400