

Original article / Araştırma

Thiol/disulphide homeostasis as a new oxidative stress marker in untreated patients with generalized anxiety disorder

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ABSTRACT

Objectives: Previous studies have investigated the role of oxidative stress in the etiology of generalized anxiety disorder (GAD). Thiols are an important component of the antioxidant buffer system. Dynamic thiol/disulphide homeostasis is a system that has a significant role in processes such as antioxidation preservation and regulation of enzymatic reactions. However, the value of dynamic thiol/disulphide homeostasis in etiopathogenesis of GAD has not been documented. The goal of this study was to determine dynamic thiol/disulphide homeostasis, which has an important role in antioxidant defense in untreated patients with GAD. **Methods:** This study included 25 patients who were diagnosed with GAD and 24 age-matched healthy controls. In both groups, thiol/disulphide homeostasis was assessed using an automated assay method. The Hamilton Anxiety Rating Scale (HAM-A) was used to determine the anxiety levels of patients with GAD. **Results:** Patients with GAD had higher total thiol and native thiol levels compared to the control group, but the differences were not statistically significant. In patients with GAD, the dynamic disulphide levels, disulphide/native thiol, and disulphide/total thiol ratios were higher compared to the control group. However, the native thiol-to-total thiol ratio was significantly lower in patients with GAD compared to the control group. Pearson's correlation analysis showed no statistically significant correlations between total HAM-A scores and oxidative stress parameter levels in the patient group. **Conclusions:** This is the first report to assess the thiol/disulphide homeostasis in GAD patients. Our study provides evidence that the disulphide-to-native thiol and disulphide-to-total thiol ratios are higher in untreated patients with GAD than in the control group. This suggests that thiol/disulfide homeostasis is impaired in GAD patients. (*Anatolian Journal of Psychiatry* 2018; 19:143-149)

Keywords: generalized anxiety disorder, oxidative stress, thiol/disulphide homeostasis

Tedavi edilmemiş yaygın anksiyete bozukluğu olan hastalarda yeni bir oksidatif stres belirleyicisi olan tiyol/disülfid homeostazi

ÖZ

Amaç: Önceki çalışmalarda, yaygın anksiyete bozukluğunun (YAB) etiyolojisinde, oksidatif stresin rolü araştırılmıştır. Tiyoller antioksidan savunma sisteminin önemli bir bileşenidir. Dinamik tiyol/disülfid homeostazi, antioksidan savunma sistemi ve enzimatik reaksiyonların düzenlenmesi gibi süreçlerde, önemli bir role sahiptir. Bununla birlikte, YAB'nin etiopatogenezinde dinamik tiyol/disülfid homeostazının önemine ilişkin herhangi bir çalışma yoktur. Bu çalışmanın amacı tedavi edilmeyen YAB hastalarında, oksidan/antioksidan dengenin önemli bir göstergesi olan dinamik tiyol/disülfid homeostazını araştırmaktır. **Yöntem:** Çalışmaya YAB'li 25 hasta ile, yaşları uyumlu 24 sağlıklı kontrol alındı. Her iki grupta, tiyol/disülfid homeostaz parametreleri, yeni ve tam otomatik bir yöntem ile analiz edildi.

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YAB'li hastaların anksiyete düzeylerini belirlemek için Hamilton Anksiyete Derecelendirme Ölçeği (HAM-A) kullanıldı. **Bulgular:** YAB olan hastaların total tiyol ve natif tiyol düzeyleri, kontrol grubuna göre daha yüksekti, ancak istatistiksel olarak anlamlı fark bulunmadı. YAB'li hastalarda disülfid düzeyleri, disülfid/natif tiyol ve disülfid/total tiyol oranları, kontrol grubuna göre daha yüksek bulundu. Bununla birlikte, natif tiyol/total tiyol oranı, YAB hastalarında, kontrol grubuna göre anlamlı derecede düşük bulundu. Hasta grubundaki HAM-A puanları ile oksidatif stres parametreleri arasında, istatistiksel olarak anlamlı bir ilişki saptanmadı. **Sonuç:** Bu çalışma, YAB hastalarında tiyol/disülfid homeostazını değerlendiren ilk çalışmadır. Çalışmamız, disülfid/natif tiyol ve disülfid/total tiyol oranlarının, tedavi edilmemiş YAB hastalarında, kontrol grubuna göre daha yüksek olduğuna ilişkin kanıtlar sunmaktadır. Bu durum YAB hastalarında tiyol/disülfid homeostazının bozulduğunu göstermektedir. (*Anadolu Psikiyatri Derg* 2018; 19(1):143-149)

Anahtar sözcükler: Yaygın anksiyete bozukluğu, oksidatif stres, tiyol / disülfid homeostazi

INTRODUCTION

Generalized anxiety disorder (GAD) is a state of extreme anxiety, fear, and anxious anticipation, which occurs almost daily with several events and activities, and which a person cannot control. There are also several symptoms such as restlessness, excitement, weakness, fatigue, difficulty in concentrating, muscular tension, and sleep disturbance.^{1,2} The prevalence of GAD in the general population is between 3% and 6%. It has been reported that it is twice as common in women as in men.³ However, the etiopathogenesis is not fully understood even though there are various psychological, social, genetic, and biological factors among the causes of GAD.³

Susceptibility of the brain to oxidative stress suggests that oxidative stress may play a role in neuropsychiatric disorders.^{4,5} It has been shown that oxidative metabolic balance is impaired in psychiatric disorders such as depression, anxiety, attention deficit hyperactivity disorder, obsessive compulsive disorder, nocturnal enuresis, and conversion disorder.⁶⁻¹⁰ Several studies have been conducted on the role of oxidative stress in the etiology of anxiety disorders.^{6,11,112}

Thiols are organic compounds containing a sulfhydryl group that can react with free radicals to provide protection against tissue and cell damage caused by reactive oxygen products. These oxygen products are found in free or oxidized form in the plasma.¹³ The human body contains large molecular weight protein thiols and low molecular weight free thiols. Glutathione, thioredoxin, and glutaredoxin are known as endogenous biothiols. Physiological conditions can affect the pro-oxidant or antioxidant effects of plasma thiols. In some studies, pro-oxidant effects of thiols have been shown to be associated with cardiovascular and cerebrovascular diseases. However, they are generally considered to be antioxidants.¹⁴ Thiol groups are inversely oxidized to form disulfide bonds in the presence of oxygen, and there is a mutual ex-

change between thiols (reduced state) and disulphide groups (oxidized state). Consequently, disulfide bonds can be reduced back to the thiol groups to maintain homeostasis between the thiol and disulfide groups.^{15,16}

Dynamic thiol/disulphide homeostasis plays a vital role in mechanisms such as antioxidant defense, regulation of enzyme activity, and cellular signal transduction pathways.^{17,18} It is indicated that the dynamic thiol/disulphide homeostasis in the organism can be influenced in disease processes caused by oxidative stress. Oxidative stress is an important factor that influences the redox state and it explains why thiol groups are metabolized to disulphide groups. Recently, a new fully automated method was developed to evaluate thiol/disulphide homeostasis.¹⁹ Serum dynamic thiol/disulphide homeostasis in patients with GAD has not been addressed. Therefore, in this study, we aimed to evaluate the dynamic thiol/disulphide homeostasis in untreated patients with GAD and to compare these GAD patients with the healthy subjects.

METHODS

Subjects

This study included 25 patients (19 females and 6 males), between the ages of 20 and 60 years (mean age, 32±11 years), who were diagnosed with GAD by psychiatrists working in at outpatient or inpatients units at the Harran University Psychiatry Clinic; the diagnoses were made using the DSM-5 structured clinical interview.²⁰ The results of all physical examinations were normal and the patients did not have other psychiatric disorders except GAD. In addition, the subjects were not receiving any drugs, not smoking or consuming alcohol, and had no known organic pathology. Duration of illness in patients with GAD was 9.48±1.41 months.

Patients with known diabetes mellitus, kidney failure, liver disease, thyroid disease, cardiovas-

cular and cerebrovascular disease, pregnancy, epilepsy, dementia, depression, panic disorders, obsessive compulsive disorder, schizophrenia, bipolar disorder, history of serious head trauma, usage of supplemental vitamins, and inadequate samplings were excluded from the study.

The control group consisted of 24 age-matched healthy subjects (16 women and 8 men), between the ages of 17 and 41 years (mean age, 29 ± 6 years), who were asymptomatic with unremarkable medical histories or normal physical examinations. Healthy control subjects were selected from the hospital staff and their relatives. The control subjects were not receiving any drugs, not smoking or consuming alcohol, and had no known organic pathology.

This study was approved by Harran University's Corporate Ethics Committee and written informed consent was obtained from all participants.

Psychiatric measurements

The Hamilton Anxiety Rating Scale (HAM-A) was used to assess the severity of anxiety in the GAD patients. The original scale was first developed by Hamilton et al.²¹ in 1959. Validity and reliability of its Turkish version was demonstrated by Yazici et al.²² in 2001. The HAM-A is composed of 14 items and provides scores between 0 and 56. An increase in the total score reflects an increased anxiety level in the subjects, which is helpful in determining the severity and symptom profile of anxiety. HAM-A was performed by an experienced psychiatrist to measure patient anxiety levels and symptom profiles.

Blood collection

For thiol/disulphide hemostasis tests, blood samples taken from participants in the patient and control groups after a 12-hour fast, and were collected using the antecubital vein. Blood samples were transferred to tubes, centrifuged at 3000 rpm for 10 min to separate serum, and stored on ice to be processed within 6 h. Serum samples were stored at -80°C until use. The biochemical analyses were conducted at the Yildirim Beyazıt University Faculty of Medicine Biochemistry Laboratory after collecting all the blood samples.

Thiol/disulphide homeostasis

Thiol/disulphide levels were measured with a newly developed, fully automated, and colorimetric method using commercial kit (Rel assay diagnostic, Turkey).¹⁹ After disulphide levels were divided into native thiol and total thiol

levels, and the disulphide-to-native thiol, disulphide-to-total thiol, and native thiol-to-total thiol ratios were calculated as percentages.

Statistical analysis

The results are expressed as the mean and standard deviation. Shapiro-Wilk tests were used to test the normality of distribution. Two-group comparisons of parametric variables were made using the Student's *t*-test. The chi-square test was used to compare sex distribution. Pearson's correlation analysis was used to determine the associations between oxidative stress parameters, HAM-A scores, and duration of illness. The results were considered to be statistically significant when the *p* value was less than 0.05. All statistical calculations were performed using the Statistical Package for the Social Sciences for Windows version 20 software (SPSS ver. 20.0, IBM Inc., Chicago, IL).

RESULTS

The demographic characteristics of all groups are presented in Table 1. The study population consisted of a total of 49 subjects, including 25 patients. Of the 25 patients, 19 were female (76%) and 6 were male (24%), and their age ranged from 20 to 60 years (mean age, 32 ± 11 years). Of the 24 healthy controls, 16 (66.66%) were female and 8 were male (33.33%), and their age ranged from 17 to 41 years (mean age, 29 ± 6 years). There were no statistically significant differences in age and sex between two groups ($p > 0.05$) (Table 1).

Native thiol, disulphide levels; the disulphide-to-native thiol ratio, disulphide-to-total thiol ratio, native thiol-to-total thiol ratios; and comparison between patient and control groups are presented in Table 2. Patients with GAD had higher the total thiol and native thiol levels, but the differences were not statistically significant (both, $p > 0.05$). In patients with GAD, the dynamic disulphide levels, and the disulphide-to-native thiol and disulphide-to-total thiol ratios were higher compared to the control group ($p = 0.045$, $p = 0.019$, and $p = 0.019$; respectively). However, the native thiol-to-total thiol ratio was significantly lower in patients with GAD than in the control group ($p = 0.020$) (Table 2).

In the Pearson's correlation analysis, there were no statistically significant correlations between oxidative stress parameters levels, HAM-A scores, and duration of illness in the patient group ($p > 0.05$).

Table 1. Demographic characteristics of the two study groups

Parameters	GAD (n=25)	Control (n=24)	p
Age (year)	32±11	29±6	0.171
Sex (male/female)	6/19	8/16	0.470
HAM-A	29.76±4.85		
Duration of illness (month)	9.48±1.41		

Values are presented as mean±SD; GAD: Generalized anxiety disorder
HAM-A: Hamilton Anxiety Rating Scale

Table 2. The mean values of total thiol, disulphide, (%) disulphide-to-native thiol, (%) disulphide-to-total thiol, and (%) native thiol-to-total thiol in both the groups

	GAD (n=25)	Control (n=24)	p
Native thiol (μmol/l)	433.1±91.9	434.7±48.8	0.941
Total thiol (μmol/l)	475.3±94.1	470.4±52.1	0.824
Dynamic disulphide (μmol/l)	21.1±5.7	17.8±5.1	0.045
Disulphide/native thiol (%)	5.03±1.64	4.04±1.14	0.019
Disulphide/total thiol (%)	4.53±1.33	3.71±0.95	0.018
Native thiol/total thiol (%)	90.92±2.66	92.53±1.90	0.020

Values are presented as mean±SD; GAD: Generalized anxiety disorder

DISCUSSION

In this study, we hypothesized that there may be an association between thiol/disulphide homeostasis and GAD etiopathogenesis. There is no previous study investigating the serum thiol levels and thiol/disulphide homeostasis in patients with GAD. Therefore, the purpose of the current study was to assess thiol/disulphide homeostasis in the GAD.

Our results revealed that dynamic disulphide levels, and disulphide-to-native thiol and disulphide-to-total thiol ratios were higher in untreated patients with GAD compared with control. However, we demonstrated no statistically significant differences in the total thiol and native thiol levels between GAD patients and healthy subjects. Additionally, the native thiol-to-total thiol ratio was significantly lower in untreated patients with GAD compared with the control group. According to published studies, this is the first report to indicate serum thiol levels and thiol/disulphide homeostasis in untreated patients with GAD.

In our current study, there was no correlation between the patients' parameters and the disease severity as determined by HAM-A in untreated patients with GAD. Similar to our study, Herken et al.²³ reported no significant associa-

tion between disease severity and oxidative stress parameters in patients with panic disorder. Emhan et al.⁶ addressed oxidative and antioxidative levels in patients with GAD.⁶ In that study, increased oxidative stress was not associated with disease severity.⁶ In the present study, we aimed to evaluate novel oxidative stress parameters such as serum thiol levels and thiol/disulphide homeostasis in untreated patients with GAD and to compare these parameters between GAD patients and healthy subjects. Thus, we were unable to compare this finding with other published results. According to our unpublished results of a recent study, this research was the first to investigate the correlation between thiol/disulphide homeostasis and disease severity in untreated patients with GAD.

Additionally, there was no published correlation between the patient parameters studied and the disease duration in untreated patients with GAD. Similarly, Herken et al.²³ found no correlation between oxidative stress parameter levels and duration of illness in patients with panic disorder. In contrast to these studies, Ersoy et al.²⁴ reported a negative correlation between sulphydryl levels and disease duration in patients with panic disorder. However, Kuloglu et al.²⁵ showed a positive correlation between oxidative stress parameter levels and duration of illness in pa-

tients with panic disorder. Thus, we are unable to compare our finding with previously published results.

The brain is highly vulnerable to reactive oxygen radicals because it is an especially oxygenated organ that use nearly one-fifth of the oxygen consumed by the body.²⁶ Normally, reactive oxygen radicals and antioxidant defense mechanisms maintain equilibrium in healthy individuals. Oxidative stress in the body occurs because of an imbalance between antioxidants and reactive oxygen radicals, and leads to various systemic disorders.²³

Although etiopathogenesis of GAD is not clearly understood, its many psychological, social, genetic, and biological factors are known. To date, many studies have reported the role of oxidative stress in pathogenesis of anxiety disorders.^{6,12,27,28} However, previously published results are inconsistent about oxidative stress in anxiety disorders. Emhan et al. investigated the oxidative and antioxidative levels in patients with GAD.⁶ They observed increased oxidative and decreased antioxidative levels in patients with GAD compared with the control group. Additionally, serum sulfhydryl levels were assessed in patients with GAD.²⁷ In that study, serum sulfhydryl levels were significantly lower in the GAD than in healthy subjects. Bulut et al.²⁸ suggested that lipid hydroperoxide levels are increased, while the activity of paroxanase is decreased in treatment-free GAD patients. In contrast to the above studies, another study reported no statistically significant difference in antioxidant enzymes levels in patients with post-traumatic stress disorder compared with healthy subjects.¹²

Thiols are important antioxidants; the sulphydryl group protects against oxidative stress. Thiols may undergo an oxidative reaction via oxidants and form disulphide bonds. A disulphide bond is a covalent bond and the linkage is also called a disulphide bridge. The formed disulphide bonds can be reduced to thiol groups, and thus, dynamic thiol/disulphide homeostasis is maintained.^{15,16} Dynamic thiol/disulphide homeostasis plays critical roles in antioxidant protection, regulation of enzymatic activity, signal transduction, and apoptosis. Recently, Erel et al.¹⁹ developed a novel, easy, practical, automated, and optionally manual spectrophotometric assay to determine thiol/disulphide homeostasis.

This is the first study that investigated serum thiol levels and thiol/disulphide homeostasis in patients with GAD using this newly developed

automated colorimetric method. This method is used to objectively evaluate the thiol/disulphide homeostasis, which is the dynamic redox system in organisms. Methods that measure only the thiol values can only provide information on the status of the antioxidant buffer system. Both thiol and disulphide can be measured using this method, and thus, it was possible to globally evaluate the thiol/disulphide balance.

In several studies, serum thiol/disulphide homeostasis was examined in acute and chronic inflammatory disorders. In those studies, levels of native and total thiols were found to be significantly low.^{29,30} A study conducted by Vural et al.²⁹ demonstrated that total thiol and native thiol levels were decreased in patients with Parkinson's disease compared to the control group. Additionally, in that study, it was demonstrated that there was an increase in disulphide-to-native thiol and disulphide-to-total thiol ratios in patients with Parkinson's disease.²⁹ A previous study revealed that there were increased dynamic disulphide levels in children with attention deficit and hyperactivity disorder. However, the difference was not statistically significant compared to the control group.³⁰

In our current study, dynamic disulphide levels, and disulphide-to-native thiol and disulphide-to-total thiol ratios are higher in patients with GAD. In addition, we observed that the native thiol-to-total thiol ratio was significantly lower in untreated patients with GAD than in the control group. However, no statistically significant differences were detected between GAD patients and healthy subjects in the total thiol and native thiol levels. We did not find any similar published results that were related to thiol/disulphide homeostasis in patients with GAD. Our result is, thus, the first publication in this area.

An important limitation of our study is the small sample sizes in both groups, and these observations must be confirmed in a larger patient sample. Second, we did not evaluate thiol/disulphide homeostasis in GAD patients who were receiving treatment in our study. Third, we did not measure the other oxidative parameters because of technical limitations. However, we reinforced our data by evaluating thiol/disulphide homeostasis, which is a novel oxidative stress marker.

This study is the first to assess thiol/disulphide homeostasis in GAD patients. The results of our study showed that thiol/disulphide homeostasis can be used in etiopathogenesis of GAD. This balance can be measured accurately using a

newly developed automated method. Additionally, the method is easily available and relatively inexpensive.

In conclusion, the present results indicated that dynamic thiol changes were considerably impaired in untreated patients with GAD, and our findings also provide evidence that the dynamic disulphide levels, and the disulphide-to-native thiol and disulphide-to-total thiol ratios are higher

in untreated GAD patients. The alterations in total thiol and native thiol levels in untreated patients with GAD were not statistically significant. We suggest that oxidant-antioxidant imbalance may contribute to etiopathogenesis in untreated GAD patients. To better understand the effects of thiol/disulphide homeostasis in GAD clinical practice, further novel studies involving larger populations are warranted.

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