INTERNATIONAL JOURNAL OF
MEDICAL BIOCHEMISTRY

DOI: 10.14744/ijmb.2022.74755
Int J Med Biochem 2022;5(3):152-157

Research Article

Thiol/disulfide homeostasis in gastritis with or without *Helicobacter pylori*

Yakup Dulgeroglu[1], Osman Bandirmali[2]

[1]Department of Medical Biochemistry, Kulu State Hospital, Konya, Türkiye
[2]Department of General Surgery, Surgical Oncology, Ondokuz Mayıs University Faculty of Medicine, Samsun, Türkiye

Abstract

Objectives: In this study, it was aimed to evaluate how thiol/disulfide homeostasis is affected in *Helicobacter pylori* positive and negative gastritis and the efficiency of thiol/disulfide levels in the diagnosis of chronic gastritis.

Methods: Seventy patients with chronic gastritis (30 *H. pylori* negative and 40 *H. pylori* positive) and 20 healthy volunteers were enrolled in the study. An upper gastrointestinal endoscopy procedure was performed on all patients. Native and total thiol were measured using a novel automatic and spectrophotometric method developed by Erel and Neselioglu. The differences between the groups and the diagnostic efficiency of the parameters were evaluated statistically.

Results: Native thiol levels decreased in the chronic gastritis group compared with the control group, whereas disulfide levels increased only in the *H. pylori* positive gastritis group compared with the control group. No statistically significant difference was found between the groups in terms of total thiol levels. According to the ROC analysis, the highest diagnostic efficiency in distinguishing the chronic gastritis group from the control group was calculated for native thiol with a 0.953 area under the curve value, 95% sensitivity, and 90% specificity.

Conclusion: It was shown that the thiol/disulfide balance was impaired in patients with chronic gastritis compared with healthy controls, and these results were interpreted as increased oxidative stress in patients with chronic gastritis. It was determined that serum native thiol levels have high diagnostic efficiency in the diagnosis of gastritis, and it can be a potential biomarker candidate for chronic gastritis.

Keywords: Chronic gastritis, disulfide, *helicobacter pylori*, native thiol, total thiol

Helicobacter pylori is a gram-negative, flagellated, microaerophilic bacterium. The bacterium is found in the gastric mucosa, and it is claimed that half of the world's population can be infected with *H. pylori*. The bacterium can colonize the stomach and cause gastritis, peptic ulcer, gastric carcinoma, and gastric lymphoma [1]. The presence of *H. pylori* results in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the gastric mucosa. Although many cells, including epithelial cells, contribute to the production of ROS and RNS, a large amount of ROS and RNS are mainly produced in neutrophils. ROS production is catalyzed by the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase, Nox) located in the cell membrane. RNS production is catalyzed by the inducible nitric oxide synthase (iNOS) enzyme. These ROS and RNS cause oxidative damage, including DNA damage in cells [2, 3]. In conclusion, ROS and RNS produced in the stomach in the presence of *H. pylori* can cause damage to gastric cells, leading to a process that starts with gastritis and continues up to gastric cancer.

Thiol groups of proteins in cells contain a sulfhydryl (-SH) group and protect the cell against oxidative damage. As a result of the reaction of thiols with oxidants (ROS, RNS), disulfide bonds are formed, and the harmful effects of ROS and RNS molecules are prevented. Thiol levels increase again as disulfide bonds are reduced to thiol groups. Thanks to these mutual reactions, thiol/disulfide balance continues at a certain level. Oxidant agents produced in larger amounts than normal physiological processes cause a decrease in thiol lev-

Address for correspondence: Yakup Dulgeroglu, MD. Department of Medical Biochemistry, Kulu State Hospital, Konya, Türkiye
Phone: +90 505 733 19 65 **E-mail:** dulgerogluyakup@gmail.com **ORCID:** 0000-0003-0695-5708
Submitted Date: March 03, 2022 **Accepted Date:** May 26, 2022 **Available Online Date:** September 14, 2022

els and an increase in disulfide levels. The deterioration of thiol/disulfide balance in favor of disulfide is interpreted as an increase in oxidative stress [4, 5].

This study aimed to determine whether the serum levels of thiol and disulfide are different in patients with *H. pylori* positive and negative gastritis compared with healthy controls. Furthermore, the study aimed to evaluate the efficiency of these parameters in the diagnosis of gastritis and oxidative stress.

Materials and Methods

Study groups

A total of 90 participants, including 20 healthy individuals and 70 patients with chronic gastritis, were included in the study. According to the results of the gastric biopsy, two groups were formed: Gastritis HP(+) (gastritis patients with positive *H. pylori*, n=40) and Gastritis HP(-) (gastritis patients with negative *H. pylori*, n=30). The control group (n=20) consisted of apparently healthy individuals who had no symptoms of gastritis, had no signs of inflammation in laboratory tests, and were compatible with the other groups in terms of age and gender. Patients diagnosed with diseases that may cause oxidative stress such as diabetes mellitus, hypertension, peptic ulcer, cancer, autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, and ankylosing spondylitis), and systemic infection (high CRP or sedimentation and leukocytosis) were not included in the study. Those who were under proton pump inhibitor or H_2 receptor blocker medication within one week prior to the study were excluded.

Written consent was obtained from the participants. The ethical approval was obtained from the Ethics Committee of KTO Karatay University, Faculty of Medicine, Türkiye (No.: 2020/024).

Sample collection

Venous blood was taken from patients who applied to Kulu State Hospital General Surgery Clinic with symptoms of gastritis in gel tubes (BD, 8 mL) without anticoagulant before diagnostic endoscopy. The blood collected was kept at room temperature for 30 min until the coagulation was completed, and serum was obtained by centrifugation at 1500×g for 10 min. The serum was aliquoted and stored at -20°C until the working day.

An upper gastrointestinal endoscopy procedure was performed on the patients, and biopsy samples were obtained. The presence of *H. pylori* in biopsy specimens was determined by pathological examination.

Measured native thiol, total thiol, and disulfide levels

An automated spectrophotometric method developed by Erel and Neselioglu [6], which is available commercially (Rel Assay Diagnostics, Türkiye), was used for the measurement of native thiol and total thiol. To determine the native thiol and total thiol levels, disulfide bonds were reduced to thiol groups using sodium borohydride ($NaBH_4$) first. The unused $NaBH_4$ was removed with formaldehyde. Native and total thiol levels were measured after reaction with 5,5´-dithiobis(2-nitrobenzoic) acid. Modified Ellman reagent was used to measure the total thiol content in the samples. To calculate the disulfide level, the result obtained by subtracting the native thiol from the total thiol was divided by 2. After determining the native thiol, total thiol, and disulfide values, native thiol/total thiol, disulfide/total thiol, and disulfide/native thiol ratios were calculated.

Statistical analysis

PASW statistics program was used for statistical analysis (Predictive Analytics Software, Version 18.0. Chicago: SPSS, Inc.). The data were evaluated using visual (histograms) and statistical methods (the Shapiro-Wilk test) to determine whether they were normally distributed. It was observed that all parameters conformed to normal distribution. Descriptive analyses were expressed as mean±SD. An ANOVA test was used to determine the differences between the groups. Tukey test was used for post hoc analysis. ROC analysis was performed to calculate the diagnostic performance of the parameters. The determination of the most appropriate cutoff value was made using Youden's index. A value of $p<0.05$ was considered statistically significant.

Results

A total of 90 participants, 38 men and 52 women, were included in the study. The mean ages of Gastritis HP(-), Gastritis HP(+), and the healthy control group were calculated as 43.73, 45.22, and 38.15 years, respectively. There was no significant difference in age between the groups (p=0.125). The laboratory results of the groups are presented in Table 1.

Serum native thiol and disulfide levels were different between the groups. In the post hoc analysis, it was determined that serum native thiol levels were significantly lower in Gastritis HP(-) and Gastritis HP(+) compared with the control group ($p<0.001$ and $p<0.001$, respectively). Although native thiol levels were lower in Gastritis HP(+) compared with Gastritis HP(-), the difference was not statistically significant (p=0.697). Serum disulfide levels in Gastritis HP(+) were significantly higher than in the control group (p=0.010). There was no significant difference between the other groups in terms of disulfide levels ($p>0.05$). Figure 1 shows the distribution of thiol/disulfide parameters in the study groups.

Native thiol/total thiol ratio was found to be lower in both Gastritis HP(-) and Gastritis HP(+) groups compared with the control group. Disulfide/native thiol and disulfide/total thiol ratios were found to be higher in both Gastritis HP(-) and Gastritis HP(+) groups compared with the control group. No significant difference was found between Gastritis HP(-) and Gastritis HP(+) groups. Statistical data on post hoc analysis between groups are presented in Table 2.

Table 1. Levels of serum thiol/disulfide parameters in the groups

Variables	Control group	Gastritis (HP-)	Gastritis (HP+)	p
Native thiol(μmol/L) (mean ±SD)	162 (±29.62)	100 (±34.07)	94 (±28.24)	0.000
Total thiol(μmol/L) (mean ±SD)	244 (±40.83)	211 (±59.18)	222 (±59.53)	0.132
Disulfide (μmol/L) (mean ±SD)	41 (±20.35)	56 (±28.29)	64 (±31.58)	0.015
Native thiol/Total thiol (% ±SD)	67 (±11.66)	49 (±15.09)	44 (±15.30)	0.000
Disulfide/native thiol (% ±SD)	27 (±14.74)	68 (±60.92)	79 (±58.56)	0.002
Disulfide/total thiol (% ±SD)	16 (±5.83)	25 (±7.55)	28 (±7.65)	0.000

P values were obtained by Oneway ANOVA test. n: number of person; SD: standard deviation.

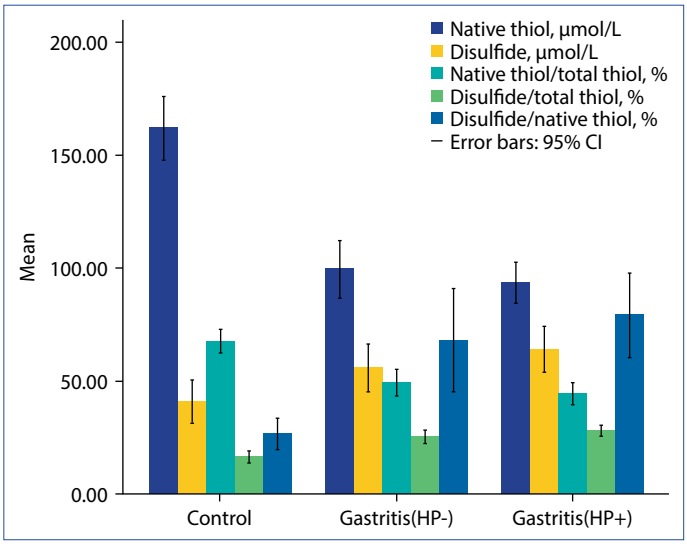

Figure 1. Distribution of thiol/disulfide parameters in the study groups.

CI: Confidence interval.

ROC analysis was performed to evaluate the diagnostic performance of the parameters in the discrimination of the groups. Accordingly, it was observed that native thiol, disulfide, and disulfide/native thiol levels have statistically significant diagnostic efficiency in the discrimination of Gastritis HP(-) and Gastritis HP(+) groups from the control group. The area under the curve (AUC), sensitivity, and specificity values calculated by ROC analysis are shown in Table 3.

Native thiol had the highest AUC value in discrimination of the groups. In the discrimination of gastritis patients from the control group, the AUC value was calculated as 0.953 for native thiol. The sensitivity and specificity of native thiol in the discrimination of the control group and patients with gastritis were calculated as 95% and 90%, respectively. While the sensitivity and specificity values of native thiol in the discrimination of the control group and Gastritis HP(-) were 95% and 87%, respectively, it was determined as 95% and 92%, respectively, in the discrimination of control group and Gastritis HP(+). The effectiveness of other parameters in distinguishing the groups from each other was found to be lower than native thiol. None of the parameters included in the study had a statistically significant AUC value in the differentiation of Gastritis HP(-) and Gastritis HP(+) groups. ROC curves of the parameters in the discrimination of the groups are shown in Figure 2.

Discussion

According to our study, while native thiol serum levels decreased in the Gastritis HP(-) and Gastritis HP(+) groups compared with the control group, it was determined that disulfide levels increased in Gastritis HP(+) versus the control group. However, it was observed that none of these parameters were significantly different between the Gastritis HP(-) and Gastritis HP(+) groups.

There is a close relationship between inflammation and oxidative stress. Although ROS and RNS production is in other cells, it mostly occurs in neutrophils with the stimulation of proinflammatory cytokines. For example, TNF-α increases ROS production in neutrophils and other cells, while IL-1-β, interferon-γ, and TNF-α increase RNS production in epithelial cells and inflammatory cells by stimulating the expression of iNOS [7]. The harmful effects of these oxidant molecules in the cell

Table 2. Post hoc analysis results between the groups

Variables	Control[a] vs Gastritis(HP-)[b]	Control[a] vs Gastritis(HP+)[b]	Gastritis(HP-)[a] vs Gastritis(HP+)[b]
Native thiol (mean difference, μmol/L)	62.45 (p<0.001)	68.45 (p<0.001)	6.00 (p=0.697)
Disulfide (mean difference, μmol/L)	-14.91 (p=0.169)	-23.11 (p=0.010)	-8.20 (p=0.458)
Native thiol/total thiol (mean difference, %)	18.19 (p<0.001)	23.00 (p<0.001)	4.81 (p=0.360)
Disulfide/native thiol (mean difference, %)	-41.38 (p=0.023)	-52.59 (p=0.001)	-11.22 (p=0.658)
Disulfide/total thiol (mean difference, %)	-9.09 (p<0.001)	-11.50 (p<0.001)	-2.40 (p=0.360)

Mean difference shows the result a-b. P values were obtained from the Tukey test.

Table 3. Diagnostic efficiency of the parameters in discrimination of the study groups

Groups	Variables	Native thiol	Disulfide	Disulfide/native thiol
Control vs Gastritis(HP-)	AUC	0.931 (p<0.001)	0.673 (p=0.039)	0.833 (p<0.001)
	Sensitivity, %	95	65	80
	Specificity, %	87	70	80
Control vs Gastritis(HP+)	AUC	0.969 (p<0.001)	0.747 (p=0.002)	0.886 (p<0.001)
	Sensitivity, %	95	65	85
	Specificity, %	92	77	84
Control vs Gastritis(HP-)+(HP+)	AUC	0.953 (p<0.001)	0.716 (p=0.003)	0.864 (p<0.001)
	Sensitivity, %	95	65	85
	Specificity, %	90	74	79

AUC: Area under the curve.

are neutralized by antioxidant molecules. These antioxidants include glutathione and thioredoxin. Reduced glutathione (GSH), catalyzed by the glutathione peroxidase enzyme, reacts with lipid peroxides or H_2O_2, eliminating their harmful effects, and transforms into disulfide form (GSSG). This oxidized glutathione is converted back into reduced glutathione by the glutathione reductase enzyme in the presence of NADPH. Similarly, reduced thioredoxin reacts with disulfides (-SS) in oxidized proteins to transform them into thiol (-SH) form. Oxidized thioredoxin is also reduced again by thioredoxin reductase in the presence of NADPH [8]. It is thought that the balance between these thiol and disulfide groups in proteins is disrupted in favor of disulfide with excessive ROS and RNS production due to inflammatory diseases such as chronic gastritis. As a result, all these processes lead to DNA damage and neoplastic transformation in cells [9]. In our study, the decrease in native thiol levels in the serum of patients with chronic gastritis shows that chronic inflammation causes oxidative stress independent of *H. pylori*. On the other hand, the increase in disulfide levels only in the Gastritis HP(+) group compared with the control group suggests that *H. pylori* makes an additional contribution to oxidative stress. Although not statistically significant, the fact that the native thiol levels in the Gastritis HP(+) group were lower than those in the Gastritis HP(-) group supports the idea that *H. pylori* contributes additionally to oxidative stress (Table 2). Due to the relatively small number of patients included in our study, we think this finding should be investigated with a larger number of patients.

Different results were obtained in two studies in which the control group consisted of patients with gastritis who were *H. pylori* negative. In a study by Baykan et al. [10], it was reported that there was no significant difference between the *H. pylori* positive gastritis group and the control group. In another study by Erdogdu et al. [11], it was reported that native thiol and total thiol levels were increased in the *H. pylori* positive patient group compared with the *H. pylori* negative patient group, while the disulfide levels were not different. Consistent with the results of our study, Kalkan et al. [12] reported that the native thiol, total thiol, and native thiol/total thiol ratios decreased in autoimmune gastritis, while the disulfide levels increased compared with the healthy control group. In our study, no statistical difference was found between Gastritis HP(-) and Gastritis HP(+) groups in terms of native thiol, total thiol, and disulfide levels, which is consistent with other studies in the literature. However, native thiol levels were lower in all chronic gastritis patients compared with the healthy control group, while disulfide levels were higher only in the Gastritis HP(+) group. Thus, it is seen that thiol groups play an active role in preventing oxidative damage during chronic gastritis, and the thiol/disulfide balance is impaired. In addition, *H. pylori* also contributes to the deterioration of this balance.

In studies investigating the thiol/disulfide balance in different diseases with chronic inflammation, it was reported that chronic inflammation disrupted the thiol/disulfide balance in favor of disulfide, and thus, the oxidative stress increased. Tuzcu et al. [13] reported that native thiol and total thiol levels decreased and disulfide levels increased in rheumatoid arthritis, a chronic inflammatory disease, compared with the healthy control group. Baykara et al. [14] reported that native thiol levels decreased and disulfide levels increased in the patient group with ankylosing spondylitis compared with the healthy control group. In a study by Nar and Calis [15], it was reported that all three levels of native thiol, total thiol, and disulfide decreased in the asthmatic group compared with the control group. Hizal et al. [16] reported that native thiol and total thiol levels decreased in the newly diagnosed gastric adenocarcinoma patient group compared with the healthy control, but the difference in disulfide levels was not significant. According to the studies mentioned above, it is seen that native thiol levels decreased in diseases with inflammation, while different results were obtained between the studies at disulfide levels.

In our study, it was observed that native thiol levels have a very high sensitivity (95%) and specificity (90%) value in distinguishing the gastritis patient group (*H. pylori* positive and negative) from the healthy control group (Table 3). Only a few studies are available in the literature on the diagnostic efficiency of thiol/disulfide levels in various diseases. In a study

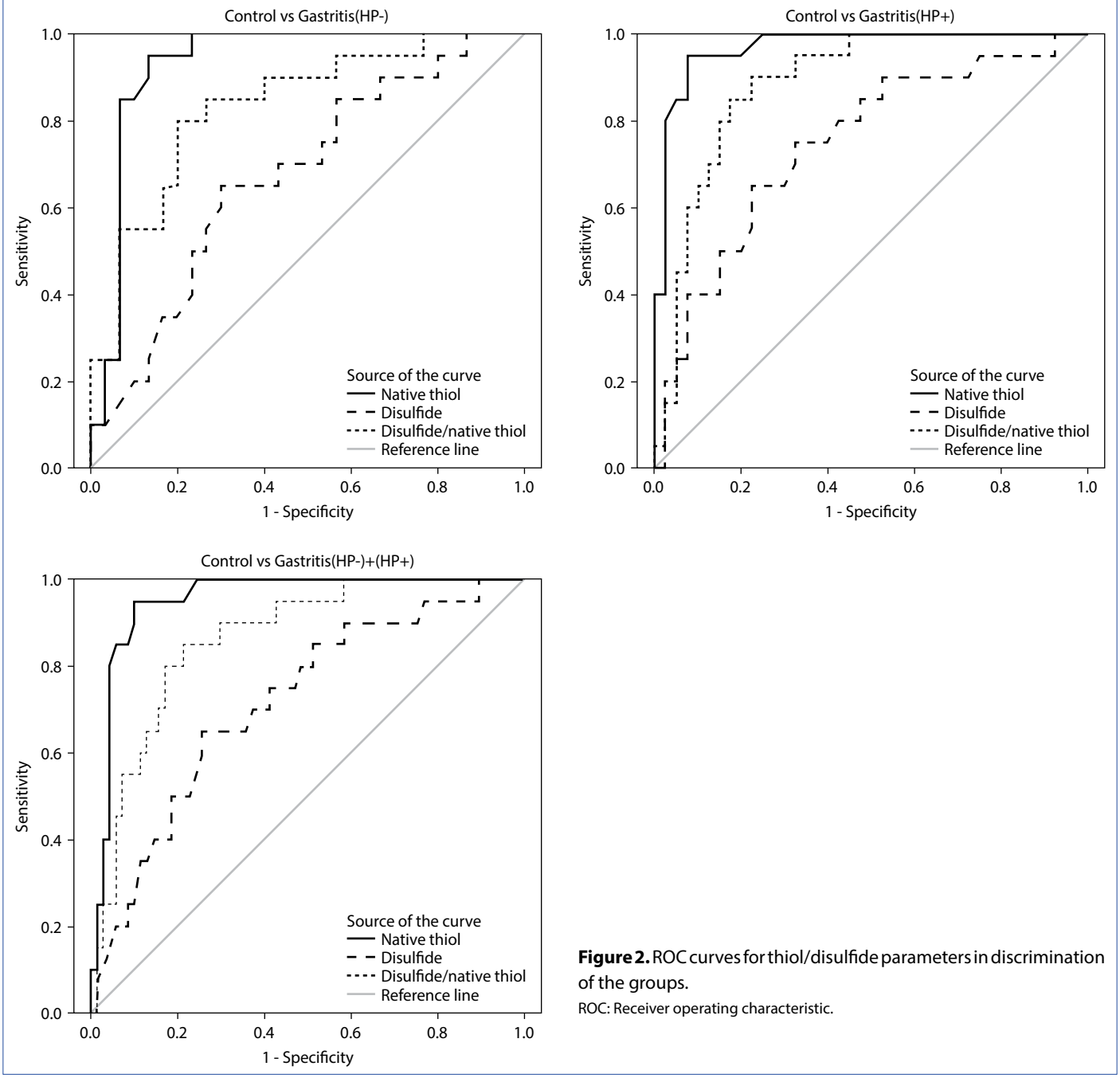

Figure 2. ROC curves for thiol/disulfide parameters in discrimination of the groups.

ROC: Receiver operating characteristic.

by Elmas et al. [17], it was reported that the sensitivity of disulfide/native thiol and disulfide/total thiol serum levels in the diagnosis of acute appendicitis in children was 86% and their specificity was 91%. Sener et al. [18] reported that the AUC value of native thiol in the diagnosis of community-acquired pneumonia was 0.895, with a sensitivity of 84% and a specificity of 85%. Kalem et al. [19] reported that the AUC value of native thiol in the diagnosis of COVID-19 was 0.962, with 97% sensitivity and 83% specificity. Considering the results of these studies in the literature and the results of our study, it is thought that thiol/disulfide homeostasis, especially native thiol, may be an important biomarker of inflammation.

The most important limitation of our study is the relatively small number of participants. The results we obtained need to be verified with a large number of participants. Another limitation is that we could not follow the patients. Determining the thiol/disulfide levels of patients diagnosed with chronic gastritis after treatment will provide clear information on whether these markers can be used in treatment follow-up.

Conclusion

In this study, it was shown that thiol disulfide homeostasis was impaired in patients with chronic gastritis compared

with healthy controls, and oxidative stress increased during chronic gastritis. Moreover, it was shown that the presence of *H. pylori* causes disulfide levels to increase in patients with gastritis, further deteriorating the thiol/disulfide balance. Also, native thiol serum levels have shown a very high sensitivity and specificity in the diagnosis of chronic gastritis. We think that thiol/disulfide balance, especially native thiol levels, may be a biomarker candidate in the diagnosis and treatment follow-up in inflammatory diseases such as chronic gastritis. However, more comprehensive studies with a large number of participants are needed in this field.

Conflict of Interest: The authors declare that there is no conflict of interest.

Ethics Committee Approval: The study was approved by The KTO Karatay University Faculty of Medicine Research Ethics Committee other than Pharmaceutical and Medical Device (No: 2020/024, Date: 24/02/2020).

Financial Disclosure: The authors declared that this study has received no financial support.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept – Y.D.; Design – Y.D.; Supervision – Y.D.; Funding – Y.D., O.B.; Materials – Y.D., O.B.; Data collection &/or processing – Y.D., O.B.; Analysis and/or interpretation – Y.D.; Literature search – Y.D.; Writing – Y.D.; Critical review – Y.D.

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