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The role of thiol/disulfide hemostasis in the diagnosis and severity prediction of acute pancreatitis

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Abstract: **Objective:** Reactive oxygen radicals are generated in the early stages of acute pancreatitis (AP) and are responsible for its progression. Thiol/disulfide homeostasis (TDH) is an important antioxidative mechanism. This study aimed to evaluate the role of TDH in the differential diagnosis of AP and predict its clinical severity.
Methods: Patients admitted to the emergency department due to upper abdominal pain were evaluated. The study consisted of two groups: the AP group and the non-AP group (patients with diagnoses other than AP). The AP group was divided into mild and severe according to acute physiology and chronic health evaluation II (APACHE-II) scores. TDH was measured with an automated assay from Erel et al. Statistical analyses were done with SPSS 16.0.
Results: The results from 128 cases—58 in the AP group and 70 in the non-AP group—were evaluated. There was no difference in TDH parameters between the AP and non-AP groups. Among the AP subgroups, native thiol (sh) and total thiol (tt) were significantly lower in the severe AP group (sh: 313.9 μmol/L, 239.1 μmol/L; tt: 351.5 μmol/L, 303 μmo/L, respectively, in the mild and severe AP groups, P-value=0.006, P-value=0.013).
Conclusion: TDH parameters change because of inflammatory processes in AP. Since this change does not occur for any specific reason, using TDH parameters for differential diagnosis of AP in patients with upper abdominal pain is not appropriate. However, in patients already diagnosed with AP, native and total thiol levels might be helpful in the prediction of clinical severity with a limited role.

Keywords: Acute Pancreatitis; Clinical Severity; Thiol/Disulfide Parameters

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1. Introduction

Acute pancreatitis (AP) is the inflammation of the pancreatic gland and is frequently seen in clinical practice with an incidence rate of 80100 individuals annually (1,2). Pathological findings may change from mild interstitial edema to severe hemorrhagic gangrene and necrosis. Mortality and morbidity might be high in severe AP cases; therefore, there are many scoring systems, such as Ranson's criteria, the acute physiologic and chronic health evaluation II (APACHE II), and the Balthazar classification, used in clinical practice to predict severity (3). However, since these scoring systems require the evaluation of many parameters, and some parameters are time dependent; a new marker is needed for the differential diagnosis and prediction of disease severity for acute pancreatitis in emergency departments. From the pathophysiological perspective, studies have demonstrated that reactive oxygen radicals, generated at very early stages of the disease, are responsible for progression of acute pancreatitis (4). Oxygen, vital for human life, can transform into harmful metabolites during metabolic processes. In normal conditions, these free oxygen radicals are removed by antioxidative systems, and oxidative stress occurs if this equilibrium is disturbed in favor of oxygen radicals. Oxidative stress may trigger many pathologic mechanisms, such as lipid peroxidation, enzymatic activation/deactivation, DNA damage, and immune system deficiency (5). Thiol, an organic component that contains sulfhydryl group (-SH), plays a vital role in antioxidative mechanisms (6). The thiol groups are oxidized by oxygen radicals and turn into reversible disulfide bond structures. These disulfide bond structures can be reduced back to thiol groups, thus maintaining thiol/disulfide homeostasis (THD). TDH can easily be measured with an automated assay, described by Erel et al. in 2014 (7). Many studies have reported on thiol levels and TDH in different diseases, such as coronary artery disease, ulcerative colitis, and AP (8). This study aimed to evaluate the role of TDH in the differential diagnosis of AP and predict the clinical severity among patients diagnosed with the disease.

2. Methods

2.1. Study design

This was a prospective study conducted in an emergency department of a tertiary hospital with the approval of the local ethics committee during a one-year period.

2.2. Study population

Patients admitted to the emergency department due to upper abdominal pain were evaluated. Among 1,121 patients, 71 of them were diagnosed with AP. Diagnosis of AP was made according to the American college of gastroenterology guidelines. The criteria levels are as follows: I, acute severe epigastric pain reflecting in the back; II, amylase/lipase levels three times greater than the standard limit; and III, characteristics showing AP upon abdominal imaging (9). Patients demonstrating two of the three criteria were diagnosed with AP. Among the remaining 1,050 patients, those diagnosed with any other diagnosis other than AP in the non-AP group of the study, were determined randomly by taking one patient from each 15, respectively. Therefore, the non-AP group consisted of 70 patients. We also evaluated the thiol/disulfide parameters of the 64 healthy volunteers as control group (Figure 1). Written informed consent was provided by all the participants or their relatives. Trauma patients, oncologic patients, patients with hematologic disorders, patients with chronic inflammatory disorders, patients younger than 18, and pregnant patients were excluded.

2.3. Study process

Demographic data, vital parameters, laboratory analysis, radiologic findings, and the outcomes of participants were recorded in prepared study forms. Among the AP group, the APACHE-II scores of the patients were calculated. The APACHE-II score is used to predict mortality for in-hospital patients. This score includes parameters of different categories. These include age, Glasgow coma scale score, vital signs (body temperature, mean arterial pressure, respiratory rate, heart rate), oxygenation status (pH, FiO2), chemistry panel (serum sodium level, serum potassium level, serum creatinine level, and presence of acute renal failure), and hematologic status (hematocrit, white blood cell count, being immunocompromised, or severe organ system insufficiency). APACHE-II scores were calculated using online medical calculation applications. Patients with APACHE-II scores 8 were grouped in the severe AP category (3).

2.4. Measurement of thiol/disulfide homeostasis

The participants' blood samples were stored at –80°C after ten minutes of centrifuge at 3600 cycles. All samples were dissolved simultaneously and studied with an automated assay developed by Erel et al. with a Roche Hitachi Cobas c501 automatic analyzer (7). This is a paired test method. Simultaneously, during the first part, the amount of native thiol groups was measured using the modified Ellman's Reagent. In the second part, dynamic disulfide bonds (-S-S-) were reduced to functional thiol groups (-SH) by sodium borohydride (NaBH4). Unused NaBH4 residues were entirely removed by formaldehyde, so extra reduction of Ellman's Reagent was prevented, and total thiol groups, both reduced and native, were accurately calculated. The disulfide bond amount was equal to half of the difference between the total thiol and native thiol amounts. The number of participants whose TDH was analyzed properly was 58 in the AP group and 70 in the non-AP group and 64 in the control group. Native thiol (sh), disulfide (ss), total thiol (tt) amounts (μmol/L), and native thiol/total thiol (sh/tt), disulfide/native thiol (ss/sh) and disulfide/total thiol (ss/tt) ratios (%) were calculated.

2.5. Statistical analysis

Statistical analyses were done with SPSS 16.0 (Chicago, IL, USA). After checking the normality distribution with the Shapiro–Wilk test, continuous variables were described as mean±standard deviation or median (interquartile range: 25–75). Categorical variables were expressed as percentages, and analyses were carried out with Pearson's chi-squared test. A student's t-test was used for parametric data, and the Mann–Whitney U test was used for non-parametric data. For >2 group comparisons Kruskal-Wallis test was used and for post hoc analysis Dwass-Steel-Critchlow-Fligner pairwise comparisons used. To calculate sensitivity and specificity deterministic 2x2 tables were used. To determine a cut-off value for the thiol/disulfide level, receiver-operating characteristic analyses were initiated, and the area under the curve (AUC) was calculated. For all analyses, P-value<0.05 was considered statistically significant.

3. Results

The results of the 192 cases are as follows: 58 in the AP group, 70 in the non-AP group and 64 in the control group were evaluated. There was no difference in terms of age and gender between the groups. When TDH parameters were evaluated between the groups, there was no difference between the groups for any of the parameters (Table 1).

To determine the cut-off level for total thiol levels, ROC analysis was made and with %81 sensitivity, 314 μmol/L tt level determined as cut-off. According to this cut-off we made diagnostic 2x2 tables. Distribution of test results were given in table 2. Based on this data sensitivity of low tt levels for diagnosis of acute pancreatitis is 39.66 % and specificity is 18.75%. For all non-healthy groups (AP + non-AP group) sensitivity of low tt level was 56.25% and specificity was 18.75%.

AP patients were divided into two groups—mild and severe. There were 13 patients in the severe AP group. TDH parameters were compared between subgroups, and, among all parameters, there were differences between sh and tt levels only, which were significantly lower in the severe AP group (Table 2). AUC was 0.73 (95% CI: 0.58,0.87) for sh and 0.732 (95% CI: 0.57,0.88) for tt (Figure 2). The ideal sh cut-off value

Table 1 Demographic findings and the groups' thiol/disulfide homeostasis parameters.

	Control group (N=64)	AP group (N=58)	Non-AP group (N=70)	P-value
Age, (year), mean±sd	51±15.3	55 ± 18.48	56 ± 19.35	0.016
Gender Male, (n), %	37.5%	37.9%	40%	0.9
Thiol/disulfide homeostasis parameters				
Native thiol (sh) (μmol/L), 95% CI	351.90 (282.22,387.07)	294.22 (229.84,358.68)	315.29 (271.87,359.39)	<0.001
Disulfide (ss) (μmol/L), 95% CI	16.97 (11.01,23.02)	19.88 (14.74,26.50)	19.20 (11.65,28.22)	0.097
Total thiol (tt) (μmol/L), 95% CI	385.35 (316.65,425.10)	335.48 (269.44,401.79)	359.85 (300.26,405.29)	0.005
Native thiol/total thiol sh/tt, (%), 95% CI	91.05 (87.94,94.13)	89.08 (83.74,91.28)	89.18 (85.80,92.54)	0.002
Disulfide/native thiol ss/sh, (%), 95% CI	4.91 (3.11,6.85)	6.12 (4.77,9.70)	6.06 (4.02,8.20)	0.002
Disulfide/total thiol ss/tt, (%), 95% CI	4.47 (2.93,6.02)	5.45 (4.35,8.12)	5.40 (3.72,7.04)	0.002*

*: Post hoc analysis showed that the difference is because of the control group. There was no difference between AP and non-AP groups; AP: Acute pancreatitis; CI: Confidence interval

Table 2 Distribution of test results between groups

	Control group (N=64)	AP group (N=58)	Non-AP group (N=70)
Test positive*	12	23	49
Test negative	52	35	21

*: Total thiol levels lower than 314 μmol/L determined as test positivity, since the negative correlation between oxidative stress and thiol levels; AP: Acute pancreatitisl

Table 3 Thiol/disulfide homeostasis parameters of patients with mild and severe acute pancreatitis

	Mild AP group (N=45)	Severe AP group (N=13)	Non-AP group
Thiol/disulfide homeostasis parameters Native thiol (sh) (μmolL1), 95% CI	313.9 (252.9,371.2)	239.1 (177.3,303.8)	0.006
Disulfide (ss) (μmolL1), 95% CI	18.7 (15.3,25.4)	21.3 (11.6,33.6)	0.84
Total thiol (tt) (μmolL1), 95% CI	351.5 (298.3,411.6)	303 (188.6,353.9)	0.013
Native thiol/total thiol sh/tt, (%), 95% CI	89.5 (83.9,91.4)	86.4 (79,89.7)	0.23
Disulfide/native thiol ss/sh, (%), 95% CI	5.8 (4.6,9.5)	7.8 (5.7,13.3)	0.23
Disulfide/total thiol ss/tt, (%), 95% CI	5.2 (4.2,8)	6.7 (5.1,10.4)	0.23

AP: Acute pancratitis; CI: Confidence interval

was 282.46 (μmol/L) with 62% sensitivity and 85% specificity (Youden's index=0.468). For tt, the ideal cut-off value was 316.2 (μmol/L) with 68% sensitivity and 85% specificity (Youden's index=0.522).

4. Discussion

Two critical results were observed in this study on the role of TDH on differential diagnosis and severity prediction of AP. First, since there was no difference in the different thiol/disulfide parameters between the groups and low sensitivity and specificity of tt for diagnosis, we believe thiol/disulfide parameters are not useful in clinical practice for differential diagnosis of patients with upper abdominal pain and diagnosis of AP. Second, since sh and tt levels were significantly lower in the severe AP patient group, despite the low AUC values, these levels may help predict the clinical severity of AP.

AP is a relatively common disease with high mortality rates

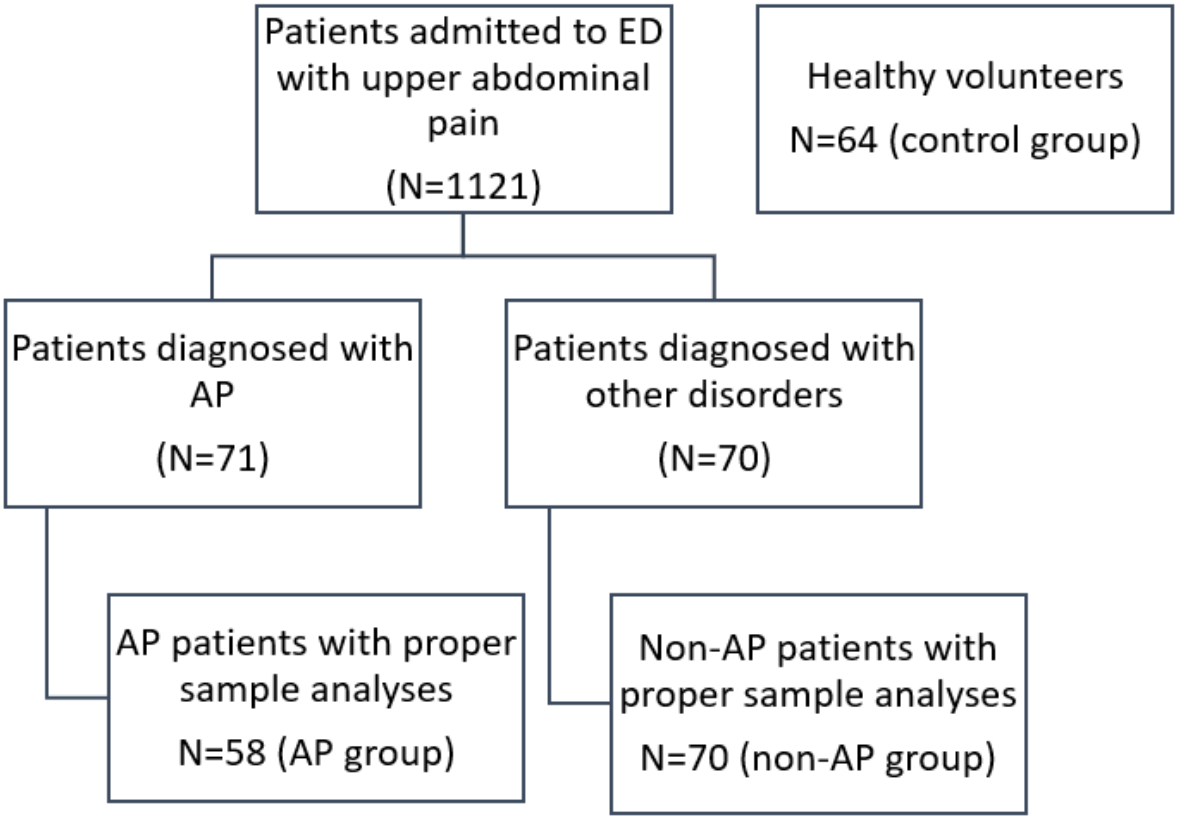

Figure 1 Flow chart. ED: Emergency department; AP: Acute pancreatitis

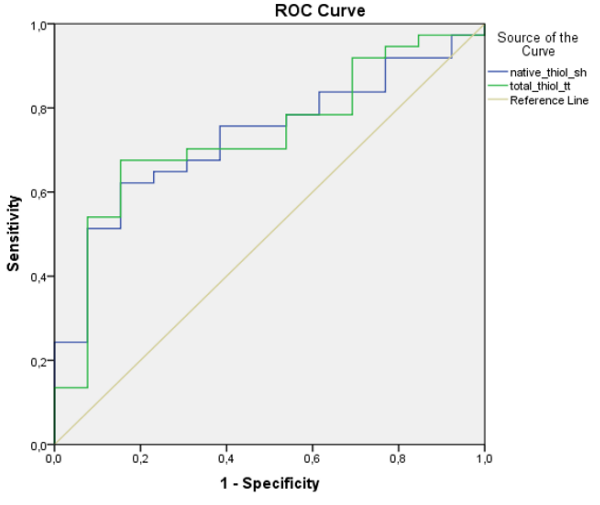

Figure 2 ROC analyses of native thiol and total thiol between mild AP and severe AP subgroups

that can reach 25% mortality in severe cases (10). The level of inflammation is the primary determinant of disease progression and clinical severity. As a consequence of inflammation, free oxygen radicals increase, and antioxidant systems are activated (11). Dynamic TDH is one of the essential antioxidant mechanisms in the body; its prognostic and diagnostic roles have been studied in a wide range of disorders, such as diabetes, inflammatory bowel disease, carbon monoxide poisoning, and various occupational disorders (12).

This study demonstrated that sh levels were significantly lower in AP patients than in healthy volunteers. Studies on thiol/disulfide parameters in AP patients have shown similar results to our study (1,6,8). However, Özyazıcı et al. studied thiol/disulfide parameters among AP patients and showed that sh levels were significantly lower in AP patients than in control group patients (13). Similarly, Kundi et al., in their study on acute myocardial infarction patients, and Ergin et al., in their study on patients with carbon monoxide poisoning, showed that sh levels decreased in patient groups compared to control groups (14,15). Therefore, these studies demonstrated that sh levels might change in every inflammatory condition independent of the main pathophysiological reason. Different from other studies, in addition to the healthy control group, we compared our results between the AP and the non-AP groups. The fact that there was no difference between AP and other diagnoses regarding TDH parameters proves that these parameters change in every inflammatory process. Therefore, we believe TDH is not useful for differential diagnosis of AP.

The correlation of thiol/disulfide parameters with the clinical severity of different disorders has previously been studied in the literature. Neselioğlu et al. demonstrated that thiol/disulfide parameters were significantly lower in pa-

tients with active ulcerative colitis compared to patients in remission (16). Similarly, Altıparmak et al. showed that lower thiol levels were associated with increased severity of coronary artery disease (17). However, Akdağ et al. observed that TDH plays a role in the pathogenesis of chronic urticaria, but they found no significant correlation between those parameters and the urticaria activity score (18). In terms of AP, studies are quite limited. Köseoğlu et al. found that the thiol/disulfide component was not correlated with the APACHE-II scores of AP patients (1). In our study, sh and tt levels were significantly lower in severe AP patients. Although its clinical use is limited due to its low AUC value to predict severity, a threshold of 261.54 (μmol/L) for sh with 73% sensitivity and 73% specificity was attained. As a result, TDH parameters has a very limited role for prediction of AP severity in clinical practice.

5. Conclusion

Thiol/disulfide parameters change as a consequence of inflammatory processes in AP. Since this change does not occur for any specific reason, using thiol/disulfide parameters for differential diagnosis of AP in patients with upper abdominal pain is not appropriate. However, in patients already diagnosed with AP, sh and tt levels may have a limited role for prediction of clinical severity. More studies with larger sample sizes are needed to determine further the role of TDH in predicting AP severity.

6. Declarations

6.1. Acknowledgement

None.

6.2. Authors' contribution

ME: Conceptualization (equal), data curation (lead), formal analysis (equal), investigation (equal), resources (equal), writing original draft (lead); GÇI: Conceptualization (equal), formal analysis (equal), methodology (equal), project administration (lead), software (equal), supervision (equal), writing review editing (lead); ÖEDV: Conceptualization (equal), methodology (equal), resources (equal); ŞKÇ; formal analysis (equal), methodology (equal), software (equal), supervision (equal), validation (equal); YÇ: Conceptualization (equal), methodology (equal), project administration (lead), resources (equal), supervision (lead), writing review editing (lead).

6.3. Conflict of interest

None.

6.4. Funding

None.

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