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Research Article

Dynamic Plasma Thiol/Disulfide Balance In Experimental Pancreatitis Model In Rats

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Abstract **Introduction:** Dynamic thiol-disulfide balance has critical roles in regulating antioxidant protection and detoxification.Thiol/disulfite levels, which can be easily measured in blood, were investigated in various proliferative and inflammatory diseases. It was observed that the changes in this balance were correlated with oxidant parameters. In previous studies, it has been shown that oxidative stress increases significantly in acute pancreatitis, and it has been shown that these levels are related to the severity of the disease. Owing to acute pancreatitis is a severe inflammatory condition; the thiol/disulfide balance may correlate with the severity of this disease. Our study investigated thiol/disulfide levels in the experimental pancreatitis model.

Materials and Methods: In our study, 16 Wistar-Albino male rats were used and divided into two groups as experimental and control groups, each group being eight rats. In the experimental group, we generated acute pancreatitis with Cerulein. Amylase, lipase, and thiol/disulfide balance were determined from inferior vena cava blood sampling with median laparotomy. **Results:** Amylase and lipase levels were found to be significantly different in the experimental group compared with the control group, but there was no significant difference in the plasma thiol/disulfide balance. **Conclusion:** The study results showed that the plasma thiol/disulfide level did not reveal the severity of acute pancreatitis. In previous studies, thiol/disulfide levels increased in oxidative stress, but this did not happen in our study. The fact that our study was conducted with a small group may have caused this situation. There is a need for more comprehensive studies to investigate in more detail the plasma thiol/disulfide balance.

Keywords Thiol/disulfide; Acute pancreatitis; Rats.

Introduction

Acute pancreatitis is inflammation of the pancreas. It is a common and non-bacterial disease characterized by the development of widespread inflammation as a result of autodigestion of the pancreas and surrounding tissue by digestive enzymes normally inactive in the pancreas, activated due to an etiological factor (1). Although it is most commonly due to gallstones, it

can also develop due to alcohol, various drugs, and excess blood lipids. Acute pancreatitis is a condition that can range from mild to mortal (2). In previous studies, it has been shown that oxidative stress increases significantly in acute pancreatitis, and it has been shown that these levels are related to the severity of the disease. However, all the methods used are complicated and lengthy methods in clinical practice. With a new method developed by Erel et al., thiol/disulfite levels, which can be easily measured in blood, were investigated in various proliferative and inflammatory diseases, and it was observed that the changes in this balance were correlated with oxidant parameters (3). A sulfhydryl group is formed when a hydrogen atom and a sulfur atom come together. Thiols are a class of organic compounds that have such sulfhydryl groups attached to a carbon atom, also known as mercaptans. When oxidation occurs, a covalent disulfide bond, called the SS bond, is formed with thiols. Oxidation of cysteine residues under oxidative stress results in mixed reversible disulfides between thiol proteins. These disulfide bonds can be reduced to thiol groups for a dynamic hemostasis. This thiol/disulfide balance plays essential roles in antioxidant protection, detoxification, enzymatic activity, regulation of transcription factors, apoptosis and cellular signaling pathways (4, 5). Owing to acute pancreatitis is a severe inflammatory condition, the thiol/disulfide balance may correlate with the severity of this disease. Our study aimed to observe thiol/disulfide balance in acute pancreatitis after induction with cerulein in rats.

Materials and Methods

Ankara Training and Research Hospital Animal Experiments Local Ethics Committee approval was obtained for this experimental study (Date: 04.06.2015, Number: 374). The study was carried out in Ankara Training and Research Hospital Experimental Animals Laboratory. 16 Wistar-Albino male rats weighing between 300-350 grams were used in the study. A total of 16 rats were divided into two groups as, experimental and control groups, each group being eight rats. Rats were held in 12-hour light and 12-hour dark cycles under suitable conditions, with four animals per cage. The rats were fed with standard feed, and their water needs were supplied with tap water. 50mcg/kg Cerulein (Sigma & Aldrich Chemie, GmbH, C-9026) was injected intraperitoneally into the rats in the experimental group. The rats in the control group were injected with 1cc normal saline intraperitoneally. After the procedure, rats were administered general anesthesia with 50 mg/kg ketamine(Ketalar vial, Parke-Davis, Eczacıbaşı Pharmaceuticals Marketing Co.. İstanbul-Turkey) and 10 mg/kg xylazine hydrochloride (Rompun vial, Bayer, Leverkusen, Germany) intramuscularly at the 30th hour. During the study, the rats were allowed to breathe

spontaneously. After the depth of anesthesia was controlled, the rats were placed under a heating lamp and placed on a heating pad in order to keep their body temperature at approximately 37 °C. A median laparotomy was performed and blood sampling was performed from the inferior vena cava. The blood samples were centrifuged at 2500 rpm for 15 minutes, and the serum samples taken were analyzed by the Roche-Hitachi-Cobas C501 autoanalyzer for amylase, lipase, AST, ALT, total bilirubin, direct bilirubin and thiol/disulfide levels **(**Table 1**).**

Statistical Analysis

There was no death in the experimental and control group animals throughout the study. The conformity of the laboratory variables in our study to the normal distribution was evaluated with the Shapiro-Wilks test. Interquartile range of the variables determined not to be normally distributed was used. The mean standard deviation values of the normally distributed variables were given. Mann-Whitney U test was used to determine whether there was a significant difference between the group variable (experimental-control) and ALP, AST and lipase variables in the study. Independent Sample T test was used to determine whether there was a significant difference between Amylase, AST, Direct bilirubin, Total bilirubin, Total thiol, Native thiol, and Disulfide variables. Boxplots and error bar graphs of the relevant variables were prepared. IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, version 21.0 Armonk, NY: IBM Corp.) was used for statistical analysis and calculations. Statistical significance level was accepted as $p<0.05$.

Results

In general, the average amylase value of the rats was 1874±308.05, while the minimum amylase value was 1407 and the maximum amylase value was 2428. In our study, the amylase average of the animals in the control group was found to be 1725±265.37, and the amylase average of the animals in the experimental group was 2023±286.59. The amylase average of the animals in the experimental group was higher than those in the control group. The amylase variable in the experimental and control groups showed a statistically significant difference (p=0.049) (Table 2, Figure 1). While the lipase median of the rats in the experimental group was 5.50 (IQR=1), the lipase median of the rats in the control group was 4.00 (IQR=1). The lipase median of the rats in the experimental group was higher than the rats in the control group. The lipase variable showed a statistically significant difference between the experimental and control groups (p=0.005) (Table 3, Figure 2).

Table 1. General descriptive statistics of variables

Parameters	Median (IQR) Mean ± SD	Min; Max
ALP	106.00 (52)	61; 382
ALT	54.50 (13)	35; 156
LIPASE	5.00 (2)	4; 7
AMYLASE	1874.19 ± 308.05	1407; 2428
AST	118.06 ± 19.28	92; 159
DBIL	0.06 ± 0.02	0.01; 0.12
TBIL	0.02 ±0.01	0.01; 0.05
TOTAL THIOL	215.12 ± 26.45	163.0; 269.8
NATİV THIOL	162.98 ± 19.78	126; 206
DISULFIDE	26.07 ±5.79	17.75; 34.65
SS/SH	16.06 ± 3.37	10.35; 22.35
SS/TSH	12.06 ± 1.95	8.57; 15.44
SH/TSH	75.87 ± 3.91	69.10; 82.84

SS/SH: Native thiol disülfit ratio, SS/TSH: Total thiol disülfit ratio, SH/TSH Native thiol Total thiol ratio

Table 2. Comparison of Amylase, AST, D.BIL, T.BIL, T.THIOL, N.THIOL, DISULFIDE, SS/SH and SS/TSH variables on a group basis

Parameters	Control group Mean ± SD	Experimental group Mean ± SD	t	p
AMYLASE	1725.12 ± 265.37	2023.25 ± 286.59	2.159	**0.049**
AST	125.25 ± 20.99	110.88 ± 15.42	1.561	0.141
DBIL	0.06 ± 0.05	0.06 ± 0.01	0.262	0.798
TBIL	0.02 ± 0.01	0.02 ± 0.01	0.000	1.000
TOTAL THIOL	213.76 ± 32.56	216.48 ±20.85	0.199	0.845
NATİV THIOL	162.80 ± 25.17	163.15 ± 14.32	0.034	0.973
DISULFIDE	25.48 ± 6.40	26.66 ± 5.48	0.398	0.696
SS/SH	15.76 ± 3.59	16.36 ± 3.36	0.347	0.734
SS/TSH	11.88 ± 2.15	12.24 ± 1.87	0.363	0.722

SS/SH: Native thiol disülfit ratio, SS/TSH: Total thiol disülfit ratio, SH/TSH Native thiol Total thiol ratio

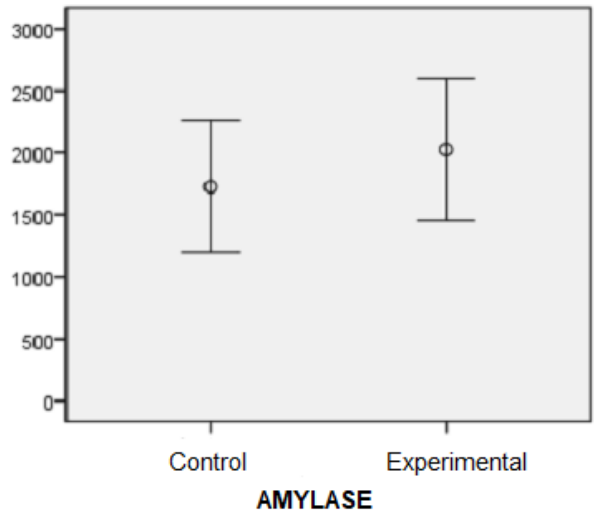

Figure 1. Comparison of amylase variable on a group basis

Table 3. Comparison of ALP, ALT, and LIPASE variables on a group basis

Parameters	Control group Median (IQR)	Experimental group Median (IQR)	Z	p
ALP	115.50 (52)	83.00 (48)	1.420	0.156
ALT	58.50 (23)	49.50 (10)	1.210	0.234
LIPASE	4.00 (1)	5.50 (1)	2.907	**0.005**

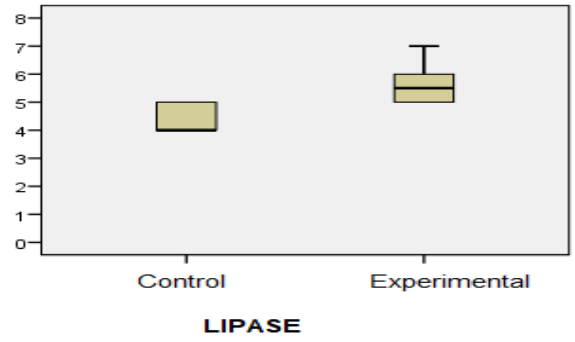

Figure 2. Comparison of lipase variable on a group basis

The disulfide of the animals in the experimental group in the study was 26.66±5.48, the disulfide of the animals in the control group was 25.48±6.40. The disulfide mean of the animals in the experimental group was higher than that of the control group, but no

statistically significant difference was found (p=0.696). While the total thiol average of the animals in the experimental group was 216.48±20.85, the total thiol average of the animals in the control group was 213.76±32.56. Total thiol mean of the experimental group is higher than that of the control group, but no statistically significant difference was found (p=0.845). The mean of native thiol of the animals in the experimental group was 163.15±14.32, and the mean of native thiol of the animals in the control group was 162.80±25.17. The native thiol average of the experimental group animals is higher than the control group animals, but no statistically significant difference was found (p=0.973). The average native thiol/disulfide ratio of the experimental group animals was 16.36±3.36, and the average of the native thiol/disulfide ratio of the control group animals was 15.76±3.59. The native thiol/disulfide ratio of the experimental group was higher than the control group, but no statistically significant difference was found (p=0.734). The mean total thiol/disulfide ratio of the animals in the experimental group was 12.24±1.87, the average of the total thiol/disulfide ratio of the animals in the control group was 11.88±2.15. The mean total thiol/disulfide ratio of the experimental group was higher than the average of the total thiol/disulfide ratio of the control group, but no statistically significant difference was found (p=0.722) (Table 2).

Discussion

Experimentally, pancreatitis was first developed by Claude Bernard in 1856 by administering olive oil to the canine pancreatic duct (6). After this study, many researchers about acute pancreatitis understand the pathophysiology and biology of the disease; conducted studies for new diagnosis and treatment options. Acute pancreatitis continues to be an important problem in surgical practice. It is believed that acinar cell damage occurs as a result of activation of zymogens in acinar cells at the beginning of acute pancreatitis (**7**). The main cause of acinar cell damage is reactive oxygen products formed as a result of inflammation. After acinar cell damage, endothelial damage, microvascular circulation disorder in the tissue, increased capillary permeability and edema occurs (8). The increase in reactive oxygen products results in an increase in oxidative stress. Under normal conditions, reactive oxygen products are neutralized by antioxidant mechanisms, but excessive and uncontrolled reactive oxygen products cause cell damage and dysfunction (9). The balance between generation and elimination of reactive oxygen products is achieved by redox sensitive signals that provide thiol and disulfide in proteins in cells under suitable conditions. In tandem with thiol oxidation due to inflammation causes an increase in reactive oxygen products and thus the progression of oxidative stres (10). Dynamic thiol-disulfide balance has critical roles in the

regulation of antioxidant protection, detoxification, signal transduction, apoptosis, enzyme activity, transcription factors, and cellular signaling mechanisms. In addition, researchers are continuing to diagnose many diseases day by day by looking at the dynamic thiol-disulfide balance. There is also evidence showing that abnormal thiol/disulfide homeostasis plays a role in the pathogenesis of various diseases including diabetes, cardiovascular diseases, cancer, rheumatoid arthritis, chronic kidney disease, acquired immunodeficiency syndrome (AIDS), Parkinson's disease, Alzheimer's disease, Friedreich ataxia (FRDA), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS). Therefore, the determination of dynamic thiol-disulfide balance can provide valuable information about various normal or abnormal biochemical processes (3). Disruption of oxidative and antioxidative balance plays an important role in the development of acute pancreatitis (11). The resulting oxidative stress is critical in the disease's severity and the development of complications that may arise (12).

Conclusion

Our study investigated the newly developed plasma thiol/disulfide balance, which shows oxidative stress in an experimental pancreatitis model. When the experimental group in which pancreatitis was induced by giving Cerulein and the control group were compared, no statistically significant difference was found between the two groups in terms of plasma thiol/disulfide balance. The fact that our study was conducted with a small group may have caused this situation. This topic should be investigated in acute pancreatitis cases with more comprehensive studies to be done.

Conflicts of interest

Authors declare no conflict of interest

Funding

This study is unfunded.

Ethical approval

Ankara Training and Research Hospital Animal Experiments Local Ethics Committee approval was obtained for this experimental study (Date: 04.06.2015, Number: 374).

References

1. Robins SL, Kumar V. Robbins Basic Pathology 7th ed. 2003.
2. Bradley EL. A clinically based classification system for acute pancreatitis: summary of the International Symposium on Acute Pancreatitis. *Arch Surg* 1993;128:586-590.
3. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem* 2014;47:326-332.
4. Koseoglu H, Alisik M, Basaran M, et al. Dynamic thiol/disulphide homeostasis in acute pancreatitis. *Turk J Gastroenterol* 2018;29:348.
5. Sen CK, Packer L. Thiol homeostasis and supplements in physical exercise. *Am J Clin Nutr* 2000;72:653S-669S.
6. Guyton AC. Textbook of Medical Physiology. 11th ed. 2006.
7. Saluja AK, Steer ML. Acute Pancreatitis-New Approaches for the Management of Acute Pancreatitis-Pathophysiology of Pancreatitis. Role of Cytokines and Other Mediators of Inflammation. *Digestion* 1999;60:27-33.
8. Mentes A. Üst gastrointestinal sistem hastalıkları 6. uzmanlık sonrası eğitim kursu kitapçığı. 2001;35-40.
9. Baser H, Can U, Karasoy D, Ay AS, Baser S, Yerlikaya FH, Ecirli S. Evaluation of oxidant/anti-oxidants status in patients with mild acute-pancreatitis. *Acta Gastroenterolog Belg* 2016;79:23.
10. Moreno ML, Escobar J, Finamor I, Martínez-Ruiz A, Sastre J. Disulfide stress and its targets in acute pancreatitis. *Inflam Allergy Drug Targets* 2014;13:312-322.
11. Dziurkowska-Marek A, Marek TA, Nowak A, Kacperek-Hartleb T, Sierka E, Nowakowska-Dulawa E. The dynamics of the oxidant-antioxidant balance in the early phase of human acute biliary pancreatitis. *Pancreatology* 2004;4:215-222.
12. Abu Zidan FM, Bonham MJ, Windsor JA. Severity of acute pancreatitis: a multivariate analysis of oxidative stress markers and modified Glasgow criteria. *Br J Surg* 2000;87:1019-1023.