EXPERIMENTAL STUDY

Role of thiol-disulfide hemostasis in early diagnosis of acute mesentery ischemia: An experimental study

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ABSTRACT

BACKGROUND: Acute mesenteric ischemia (AMI) is a rarely observed acute abdominal disease that may be mortal and is difficult to diagnose early. The aim of our study is to assess the role of Thiol-Disulphide Haemostasis (TDH), a new method for AMI which still has no specific biochemical markers for early diagnosis, and to assess it together with Ischemia-Modified Albumin (IMA) which has previously proven reliability for AMI.

METHODS: The study included 32 Wistar albino rats in four groups. The 1st group (n=8) was the control group, 2nd group (n=8) was the sham group, 3rd group (n=8) had 3 h of arterial mesentery ischemia and the 4th group (n=8) had 6 h of arterial mesentery ischemia. TDH, IMA, and serum lactate values were measured at h 0, 1, 3, and 6.

RESULTS: In the 3rd and 6th h, serum total thiol and native thiol values significantly reduced ($p<0.001$), while serum disulfide, IMA, and lactate values clearly increased ($p<0.001$). Serum thiol values were observed to reduce from the 1st h.

CONCLUSION: TDH changes in the early period of AMI. The TDH parameters can be used with IMA as diagnostic parameters for patients with suspected AMI in the early period.

Keywords: Acute mesentery ischemia; disulfide; Hemostasis; thiol.

INTRODUCTION

Acute mesenteric ischemia (AMI) is a rarely observed mortal acute abdominal disease. Mortality rates vary from 58% to 80%. Currently, it is still difficult to diagnose AMI in the early period.[1] AMI may develop with several pathological mechanisms which disrupt vascular perfusion; these include arterial emboli, arterial thrombosis, venous thrombosis, and non-occlusive mesenteric ischemia.[1–3]

Renin-angiotensin activation, vasospasm, and hypoxia develop as a result of the development of pathophysiological hypoperfusion in AMI. These are followed by apoptotic cell death, and loss of cellular villi in the intestinal mucosa. This situation causes destruction of the epithelial barrier function. Then, the defensive barrier preventing microorganisms, endotoxins, and metabolites from passing into systemic circulation is removed. The bacterial products formed and toxic metabolites pass into systemic circulation through the portal circulation route. Necrosis in the intestinal loops causes perforation. During this pathophysiological process, physical examination findings cannot be identified without the development of peritoneal irritation findings.[4,5]

Superior mesenteric artery (SMA) occlusion is generally observed as sudden onset abdominal pain accompanied by vom-

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iting and bloody diarrhea, especially in elderly patients. Physical examination findings were incompatible with severe abdominal pain and the presence of a cardiac pathology such as accompanying atrial fibrillation lead to consideration of SMA embolism. However, clinical findings are nonspecific. Occlusion of the SMA in the early period may be very difficult to diagnose biochemically.[6–8] Early diagnosis of AMI reduces mortality and morbidity. Unavoidable causes of death are multiorgan failure, sepsis, and shock in patients with delayed diagnosis.[7–9]

There is no specific biochemical marker that can be used in practical application for the early diagnosis of AMI. Current clinical practice still diagnoses mesenteric ischemia accompanied by traditional methods such as anamnesis, physical examination, routine biochemical blood tests, and imaging methods. The available radiological method of intravenous contrast mesenteric CT angiography is very specific to show venous occlusion.[1,10] In recent times, D-lactate, intestinal fatty acid-binding protein, ischemia modified albumin (IMA), α-glutathione S-transferase (α-GST), and citrulline have been studied, with diagnostic efficacy for AMI shown for these biochemical markers.[4,5]

Situations that may cause tissue ischemia such as hypoxia and hypoperfusion cause reactive oxygen species (ROS) to form. ROS are molecules causing lipid peroxidation and leading to nucleic acid and cellular damage. The first marker of protein oxidation of ROS induces oxidation of amino acids containing sulfur in disulfide groups. Thiols enter reactions with the aim of detoxification against tissue damage induced by ROS. As oxidative stress develops, the consumption of thiols for detoxication increases. Thiol protein groups are oxidized by oxygen molecules gaining a reversible transformation to disulfide bonds. When the pathology triggering oxidative stress resolves, these disulfide bonds transform back to thiol groups. The measurement method for this delicate balance is thiol-disulfide hemostasis (TDH). IMA is a marker formed by oxidative stress.[11,12] IMA is a new marker studied to show α-GST oxidative stress. The usability of IMA and α-GST for early diagnosis of AMI was proven in earlier studies.[4–6,9]

Measurement of TDH equilibrium may be beneficial for diagnosis and monitoring of patients with AMI, considering its easy applicability in the early ischemic process and sensitivity to oxidative stress.

Our aim in this study is to assess the role of TDH, a new method for AMI with no specific biochemical marker for early diagnosis, along with IMA with reliability for AMI proven previously.

MATERIALS AND METHODS

Study Protocol

The project was planned as an experimental animal study. Ethics committee permission was granted by Necmettin Erbakan University KONÜDAM Experimental Medicine Application and Research Centre (number: 2018/029). The study used 32 adult Wistar albino rats weighing 360–390 g. Animals were housed in rooms with standard conditions (22°C, 51% humidity, 12-h night-day cycles, and air exchange 12 times/h). All animals were fed and given water ad libitum.

These groups were
- 1st group (n=8): control group,
- 2nd group (n=8): sham group,
- 3rd group (n=8): 3 h arterial mesentery ischemia
- 4th group (n=8): 6 h arterial mesentery ischemia.

Surgical Procedure

Rats were fasted overnight before the experiment. Before the procedure rats were administered anesthesia with 40 mg/kg ketamine (Ketalar, Pfizer Ltd. company, İstanbul, Turkey) and 10 mg/kg xylazine (Rompun, Bayer, İstanbul, Turkey) intraperitoneal. Hairs were shaved from the abdominal region of rats. Abdominal skin had antisepsis ensured with Betadine® solution. All rats undergoing surgical procedures had a nearly 4 cm abdominal midline incision made. All procedures were completed under sterile conditions.

1st group: This was the control group and rats in this group had no surgical procedure performed. Blood of 1.2 cc was taken at 0, 1, 3 and 6 h.

2nd group: Before the procedure at 0 h, 1.2 cc blood sample was taken. Rats in the sham group were shaven on the midline. The midline was cleaned with povidone-iodine and midline laparotomy was performed. The SMA was located and palpated. The intestines were replaced in normal anatomic position. After laparotomy, the peritoneum and abdominal wall were closed with 3/0 silk sutures. In the 1st and 3rd h, 1.2 cc blood samples were taken. In the 6th h, laparotomy was performed. All intestines were assessed. Blood of 1.2 cc was taken from the vena cava inferior. Samples were taken from the small intestines for histopathologic investigations. Specimens were placed in 10% formaldehyde solution.

3rd group: Before the procedure (0 h) 1.2 cc blood sample was taken. Rats in this group were shaved along the midline. Abdominal skin was cleaned with povidone-iodine and midline laparotomy was performed. The SMA was dissected and tied with 3/0 silk. The peritoneum and abdominal wall were closed with 3/0 silk. A venous blood sample of 1.2 cc was taken in the 1st h. In the 3rd h, laparotomy was performed again in rats in this group and 1.2 cc blood was taken from the vena cava inferior. Ischemic intestinal loops were excised for histopathological investigation. Samples were placed in 10% formaldehyde solution.

4th group: Before the procedure (0 h) 1.2 cc blood sample was taken. Rats in this group were shaved along the midline.

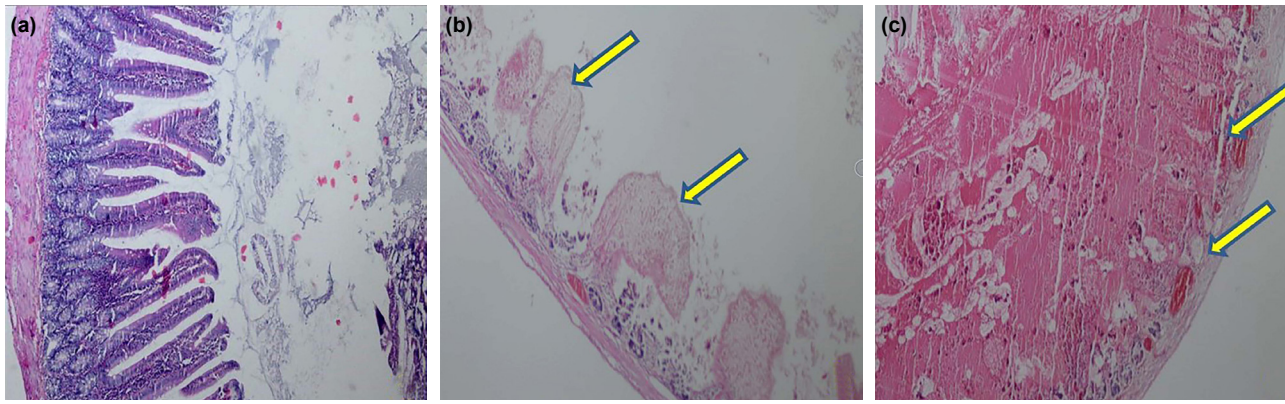

Figure 1. Histopathological examination images in rats (×40 H&E). **(a)** Normal small bowel image, **(b)** 3rd h mucosal ischemia, **(c)** 6th h full-thickness necrosis.

Abdominal skin was cleaned with povidone-iodine and midline laparotomy was performed. The SMA was dissected and tied with 3/0 silk. The peritoneum and abdominal wall were closed with 3/0 silk. A venous blood sample of 1.2 cc was taken in the 1st h. In the 6th h, laparotomy was performed again in rats in this ischemic group and 1.2 cc blood was taken from the vena cava inferior. Ischemic intestinal loops were excised for histopathological investigation. Samples were placed in 10% formaldehyde solution.

After all tissue samples were fixated in formaldehyde, routine pathological investigation was performed in paraffin blocks. Sections had 4 µm thickness. Hematoxylin and eosin staining were performed and preparates were investigated. Histopathological investigation assessed as follows; grade 0: normal mucosa, grade 1: expansion in the subepithelial area, grade 2: subepithelial congestion expanding toward the villus base, grade 3: subepithelial congestion and widespread ulceration at the apex of some villi, grade 4: ulceration in villi, expanded capillary veins in the lamina propria, and grade 5: hemorrhage, ulceration, and irregularity in lamina propria.[13]

Lactate measurement was performed with a Roche vitreous chemistry 950 autoanalyzer.

Blood samples taken from all groups were centrifuged. Supernatants were removed and stored at −80° until analysis.

Biochemical serum IMA, total thiol (TT), native thiol (NT), total antioxidant status (TAS), and total oxidant status (TOS) parameters were measured with automatic spectrophotometric tests (Rel Assay Diagnostics, Turkey). Dynamic disulfide (SS) values were calculated.[13,14] Serum IMA, TT, NT, SS, TAS, and TOS values were reported as ABSU, µmol/L, µmol/L, µmol/L, mmolTrolox equivalent/L, and µmol H_2O_2 Equivalent /L, respectively. OSI was calculated with a formula (OSI = TOS (µmol H_2O_2 equivalent/L)/TAS (µmol Trolox equivalent/L) ×100).[15,16]

The Jamovi project (2020 Version 1.2 Computer Software) program was used for statistical analysis. Variables were assessed with the Anova and Independent Sample T-tests. The relation between the variables was evaluated with Pearson correlation tests. P<0.05 was accepted as statistically significant.

RESULTS

No rats died during the planned duration of the study. There were no macroscopic findings in the sham group with laparotomy performed. In the group with 3 h ischemia, intestines had brown-gray appearance. In the 4th group, re-laparotomy at the end of the 6th h observed intense ischemic odor and the color of the small intestine appeared to be brown-black and green-black with necrosis. Histopathologic investigation of rats in the 3rd group observed the ischemia degree was grade 4 for 6 rats and grade 5 for 2, while in the 4th group there was grade 4 in 2 rats and grade 5 in 6 rats (Fig. 1).

Serum TT and NT values reduced in the 1st h compared to basal values, but this was not significant. Similarly, SS, IMA, and lactate values increased in the 1st h compared to basal values, but the increase was not statistically significant. There were no statistically significant differences in the 1st h in terms of serum SS, TT, NT, IMA, and lactate values in all groups (Table 1).

In the 3rd h, the control, sham and ischemia groups had TT of 311±46, 309±50.5, and 222±23.3 µmol/L (p<0.001), and NT 287.4±49, 289±54 and 175±21.4 µmol/L (p<0.001), respectively. TT and NT values were clearly reduced in the 3rd h ischemia group. Serum disulfide values were 10±2.9, 11.3±4.2 and 23.6±3.8 µmol/L (p<.001) and IMA values were 50.6±3.62, 49.3±6.4 and 59.6±6 ABSU, respectively, with values observed to clearly increase. Serum lactate values were 0.49±0.1, 0.48±0.08 and 1.7±0.08 mmol/L and were identified to increase in the 3-h ischemia group (p<0.001) (Table 2).

In the 6th h, control, sham and ischemia groups had TT of 312.2±45.2, 310±51 and 188±15.4 µmol/L and NT of 292±48, 288±52.5 and 121±15.5 µmol/L (p<0.001), respectively, with TT and NT clearly reduced in the 6-h ischemia

Table 1. Analyses of TDH parameters, IMA and lactate values between all groups at 0 and 1st hours

Parameters	0. hour					1. hour				
	I	II	III	IV	p*	I	II	III	IV	p*
TT, µmol/L	309±46	306±50.3	304±50.3	312±48.7	.860	308.8±46	305±50.5	297±36.9	305±41.1	.870
NT, µmol/L	289±47.5	285±53.3	289±35.1	292±45.5	.840	290±47	282±54.0	275±36.5	278 ±36.5	.746
SS, µmol/L	9.9±2.9	10±4	9.6±2.37	10.3±3.9	.943	9.9±2.9	11.3±4.23	10.7±2.59	12.3±3	.677
Index 1, %	3.24±1.2	3.5±1.7	3.1±0.75	3±0.6	.921	3.2±1.7	3.81±1.74	3.6±0.1	3.6±0.1	.575
Index 2, %	3.5±1.4	3.81±2.00	3.4±0.84	3.2±0.7	.968	3.51±1.35	4.20±2.12	3.9±1.2	3.9±1.2	.611
Index 3, %	93.6±2.6	93.1±3.53	93.6±1.42	93.5±2	.964	93.5±2.6	92.4±3.7	92.7±1.9	92.7±1.9	.65
TAS	0.28±0.08	0.3±0.09	0.3±0.2	0.3±0.1	.706	0.27±0.09	0.3±0.08	0.25±0.26	0.28±0.26	.188
TOS	5.1±2.4	5.93±2.51	5.03±2.5	5.5±2.2	.036	5±2.3	5.87±2.47	5.2±5.3	5.6±5.4	.075
OSI	1.7±0.7	1.9±0.6	1.68±0.8	1.7±0.8	.605	1.83±0.7	1.89±0.6	2.3±1.41	2.35±1.3	.859
IMA, ABSU	50.8±3.8	49.9±6.6	50.8±3.7	47.9±3.9	.975	50.6±3.62	48.7±6.16	51.6±5.62	51±5.5	.587
Lactate, mg/dl	0.5±0.1	0.53±0.1	0.49±0.1	0.5±0.1	.820	0.48±0.1	0.48±0.09	0.52±0.1	0.55±0.2	.84
pH	7.37±0.02	7.37±0.03	7.36±0.03	7.37±0.02	.393	7.37±0.02	7.34±0.02	7.34±0.02	7.32±0.02	.29
HCO3, mEq/L	21.9±1.3	21.8±1.3	21.8±1.3	22±1.2	.992	21.8±1.3	21.8±1.3	20.5±1.3	20.2±1.3	.99
BE, mmol/L	0.7±1.6	0.8±1.5	0.62±1.54	0.8±1.5	.831	0.73±1.5	0.9±1.46	0.58±1.5	0.58±1.5	.83

Mean±SD, *Independent Sample T Test, Anova Test, I: Control Group, II: Sham Group, III: 3rd hour arterial mesentery ischemia group, IV: 6th hour arterial mesentery ischemia group. TT:Total thiol; NT: Native Thiol; SS: Disulphide. Index 1: Disulphide/Total thiol; Index 2: Disulphide/Native thiol; Index 3: Native Thiol/Total Thiol; AMI: Acute mesenteric ischemia; TDH: Thiol disulphide haemostasis; IMA: Ischemia modified albumine; TAS: Total antioxidant status (µmol Trolox equivalent/L); TOS: Total oxidant status (µmol H2O2 equivalent/L); OSI: Oxidative stress index; HCO3: Standard bicarbonate; BE: Base excess.

Table 2. Analyses of TDH parameters, IMA and lactate values between all groups at 3rd hour

Parameters	3rd hour			
	I	II	III	p*
TT, µmol/L	311±46	309±50.5	222±23.3	<.001
NT, µmol/L	287.4±49	289±54	175±21.4	<.001
SS, µmol/L	10±2.9	11.3±4.2	23.6±3.8	<.001
Index 1, %	3.24±1.2	3.8±1.7	10.6±1.7	<.001
Index 2, %	3.5±1.4	4.2±2.1	13.6±2.7	<.001
Index 3, %	93.5±2.5	92.4±3.7	78.6±3.4	<.001
TAS	0.26±0.09	0.3±0.08	0.26±0.3	.005
TOS	5.1±2.3	5.8±2.4	5.9±2.5	.052
OSI	1.84±0.9	1.87±0.7	3.94±2.3	<.001
IMA, ABSU	50.6±3.62	49.3±6.4	59.6±6	<.001
Lactate, mg/dl	0.49±0.1	0.48±0.08	1.7±0.08	<.001
pH	7.37±0.02	7.34±0.03	7.23±0.03	<.001
HCO3, mEq/L	21.9±1.3	21.8±1.2	16.8±1.6	<.001
BE, mmol/L	0.7±1.6	0.88±1.4	-5.7±1.54	<.001

Mean±SD, *Independent Sample T Test, Anova Test. I: Control Group, II: Sham Group, III: 3rd hour arterial mesentery ischemia group. TT: Total thiol; NT: Native Thiol; SS: Disulphide; Index 1: Disulphide/Total thiol; Index 2: Disulphide/Native thiol; Index 3: Native Thiol/Total Thiol; TDH: Thiol disulphide haemostasis; IMA: Ischemia modified albumine; TAS: Total antioxidant status (µmol Trolox equivalent/L); TOS: Total oxidant status (µmol H2O2 equivalent/L); OSI: Oxidative stress index; HCO3: Standard bicarbonate; BE: Base excess.

Table 3. Analyses of TDH parameters, IMA and lactate values between all groups at 6th hour

Parameters	6th hour			
	I	II	IV	p*
TT, mol/L	312.2±45.2	310±51	188±15.4	<.001
NT,µmol/L	292±48	288±52.5	121±15.5	<.001
SS, µmol/L	10±2.9	11.1±4.23	33.4±2.9	<.001
Index 1, %	3.3±1.2	3.8±1.74	17.9±2	<.001
Index 2, %	3.5±1.4	4.15±2.12	28±4.7	<.001
Index 3, %	93.5±2.6	92.4±3.7	64.3±3.8	<.001
TAS	0.29±0.09	0.31±0.08	0.15±0.1	<.001
TOS	5.2±2.3	5.92±2.47	6.5±2.7	.349
OSI	1.85±0.7	1.91±0.08	5.8±2.9	<.001
IMA, ABSU	51±3.7	48.7±6.4	78.8±7.2	<.001
Lactate, mg/dl	0.35±0.1	0.5±0.09	4.01±0.08	<.001
PH	7.37±0.02	7.34±0.02	7.02±0.06	<.001
HCO3, mEq/L	21.9±1.3	22±1.3	10.1±0.1	<.001
BE, mmol/L	0.73±1.5	1±1.46	-13±2.3	<.001

Mean±SD, *Independent Sample T Test, Anova Test. I: Control Group, II: Sham Group, IV: 6th hour arterial mesentery ischemia group; TT: Total thiol; NT: Native Thiol; SS: Disulphide; Index 1: Disulphide/Total thiol, Index 2: Disulphide/Native thiol, Index 3: Native Thiol/Total Thiol; TDH: Thiol disulphide haemostasis; IMA: Ischemia modified albumine; TAS: Total antioxidant status (µmol Trolox equivalent/L); TOS: Total oxidant status (µmol H2O2 equivalent/L); OSI: Oxidative stress index; HCO3: Standard bicarbonate; BE: Base excess.

group. Serum disulfide values were 10±2.9, 11.1±4.23 and 33.4±2.9 µmol/L (p<0.001) and IMA values were 50.6±3.62, 49.3±6.4 and 59.6±6 ABSU (p<0.001), respectively and values were observed to clearly increase. Serum lactate values were 0.35±0.1, 0.5±0.09 and 4.01±0.08 mmol/L, respectively, and were identified to increase in the 6-h ischemia group (p<0.001) (Table 3 and Fig. 2).

There were strong correlations observed between the TDH parameter with IMA and serum lactate value (p<0.001). While there were negative correlations between TT, NT, and serum lactate values, there were positive correlations between disulfide, IMA, and serum lactate levels (Table 4).

Table 4. Analyses of correlations between TDH parameters and Lactate, IMA, OSI

Parameters	Lactate		OSI		IMA	
	r	p*	r	p*	r	p*
TT, mol/L	-0.543	<.001	-0.536	<.001	-0.648	<.001
NT, µmol/L	-0.623	<.001	-0.617	<.001	-0.718	<.001
SS, µmol/L	0.677	<.001	0.671	<.001	0.724	<.001
Index 1, %	0.708	<.001	0.702	<.001	0.757	<.001
Index 2, %	0.713	<.001	0.711	<.001	0.740	<.001
Index 3, %	-0.689	<.001	-0.679	<.001	-0.679	<.001
IMA, ABSU	0.655	<.001	0.689	<.001	–	–

*Pearson's Correlation Test. TT: Total thiol; NT: Native Thiol; SS: Disulphide; Index 1: Disulphide/Total thiol; Index 2: Disulphide/Native thiol; Index 3: Native Thiol/Total Thiol; TDH: Thiol disulphide haemostasis; IMA: Ischemia modified albumine; OSI: Oxidative stress index.

DISCUSSION

AMI is the development of ischemic status linked to sudden hypoperfusion of the intestines as a result of sudden cessation of blood perfusion in mesenteric vascular veins. The most fre-

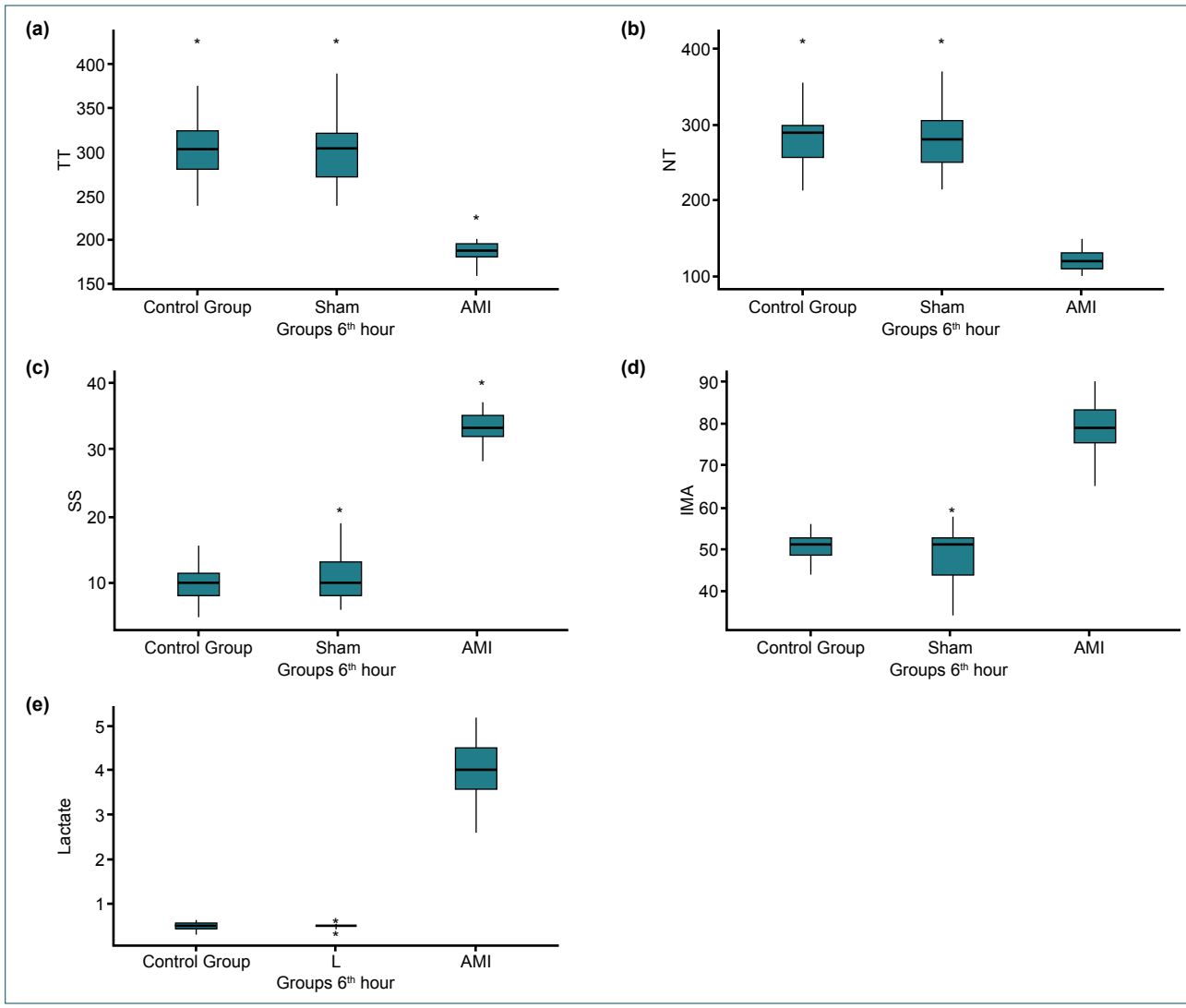

Figure 2. These plots show values of **(a)** serum TT (µmol/L), **(b)** NT (µmol/L), **(c)** SS (µmol/L), **(d)** IMA (ABSU), **(e)** lactate (mmol/L) in rats with control, sham and AMI group at 6th h. TT: Total thiol; NT: Native thiol; SS: Disulphide; IMA: Ischemia modified albumin; AMI: Acute mesenteric ischemia.

quent cause of SMA occlusion is embolism. Abdominal pain developing in patients with heart disease anamnesis should lead to the consideration of AMI.[1,3] Though AMI is rarely observed among abdominal emergencies, it is a disease with high mortality and morbidity. In the early period when occlusion begins, the physical examination may observe incompatible abdominal pain. Non-specific symptoms such as nausea and vomiting may be observed. Loss of intestinal sounds does not lead to consideration of definite AMI. Diagnosis is made more difficult by these non-specific symptoms and findings and by the most disease being observed in the early period. Early diagnosis of AMI reduces mortality and morbidity. Laboratory tests may show leukocytosis, metabolic acidosis, phosphorus elevation, LDH, and aspartate aminotransferase elevation in AMI, but these may rise in most abdominal pathologies causing peritonitis.[17,18] As a result, these tests are not specific to AMI. Studies about biomarkers to ease early diagnosis still continue.[7,19–21]

In general surgery practice, the first radiological method used for patients attending with acute abdominal pain is abdominal radiography. Most of the time there is no diagnostic value in the early period. Doppler ultrasonography may show proximal complete occlusion. However, this is not a method used in emergency conditions in routine practice. Mesenteric CT-angiography is very diagnostic for AMI linked to SMA occlusion. This method may differentiate embolism and thrombosis in mesenteric venous structures. Especially, it shows venous structures and occlusion localization before intervention for interventional radiologists, especially. These methods are contrast-dependent and expensive tests and are not performed in most hospitals.[17,22,23] We think, there is a need for lower cost, rapid, reliable biochemical methods with high diagnostic value that can be studied in primary health organizations under emergency conditions for patients considered to have AMI.

The dynamic TDH balance undertakes an important role in antioxidant protection and detoxification mechanisms. More than 50% of physiological serum antioxidant capacity comprises thiols in normal healthy individuals. Erel and Neselioglu revealed that TDH parameters were easy and applicable with a new method to calculate this balance. In this method, serum NT and TT levels are measured and disulfide levels are calculated.[16,24] IMA is easily and cheaply measured with laboratory tests of albumin-cobalt binding and enzyme-linked immunosorbent assay methods. The metal-binding capacity of human serum albumin reduces as a result of loss or change in the N-terminal binding region of albumin in acute hypoxic and ischemic situations. The measurement of this reduced metal binding capacity is given by IMA.[5,19] In the literature, IMA is found to be high in ischemic pathologies such as pulmonary embolism, acute coronary syndrome, ischemic cerebrovascular diseases, and AMI. IMA is a reference marker for ischemia in AMI.[1,5,19] Studies measuring IMA experimentally in AMI, have shown that IMA diagnostically increases in the 3rd and 6th h after SMA occlusion.[17,19] In our study, the IMA value was identified to be high in the ischemia groups after 3 and 6 h. This result is consistent with previous studies. The increase in IMA confirms oxidative stress linked to hypoperfusion in AMI. TDH parameters vary in the direction of ischemia, consistent with oxidative stress formed by AMI. Analysis of TDH and IMA parameters studied in serum can be easily performed. Today, TDH parameters are not in the hospital laboratories, because they are not a routine parameter in hospitals. This situation is a limitation for these tests at the moment. In the future, we believe they can be easily applied for patients considered to have AMI under emergency conditions other biomarkers with together.

Reduced glutathione is the most important thiol with detoxification duties in mammalian cells against ROS forming in situations such as hypoxia and hypoperfusion. Glutathione-S-transferase is the most important enzyme acting on the transformation of glutathione in the detoxification intervention against ROS. α-GST has been shown to play an active role in the detoxification of liver and small intestine cells.[14,24] Based on this principle, previous studies have shown that α-GST has high sensitivity and specificity for early diagnosis of AMI due to glutathione acting on detoxification and conjugation of small intestine cells.[9,25] In our study, TDH levels began to change in the ischemia group from the 1st h. TDH values in the ischemia groups continued to change in favor of oxidative stress in the 3rd and 6th h supporting ischemia. The reduction in serum thiol values shows that the antioxidant capacity reduced against ROS formation. As a result, TDH may show ischemia in the early period with this delicate balance of TT amounts in the human body.

Our study is the first to analyze AMI in terms of TDH and IMA parameters. In studies where AMI was experimentally measured by IMA, it was shown to increase in the 3rd and 6th h after SMA occlusion.[17,19] In our study, IMA values were compatible with previous studies. In terms of TDH, serum thiol values reduced compared to basal values in the 1st h, while serum disulfide values appeared to increase. These findings show that the oxidative balance begins to change in favor of ischemia in the early period. In the 3rd and 6th h, the reduction in serum thiol values and clear elevation in disulfide values support the view that ischemia clearly formed in tissues with increased oxidative stress. Ischemia was proven histopathologically in small intestine tissues in the 3rd and 6th h.

Serum lactate values provide information about disease prognosis in tissue hypoperfusion and ischemia. A marker of effective resuscitation in shock situations with hemodynamic instability, serum lactate values initially rise and then fall.[26,27] Metabolic acidosis and serum lactate elevation are observed in AMI. L-lactate forms within cells during the hypoxic process as only L-lactate dehydrogenase is found in mammals. The increase in serum lactate level is reported to be correlated with the amount of ischemia formed in tissue.[28] Serum lactate level measured with arterial blood gas analysis is stated to be a more valuable diagnostic parameter in the literature for arte-

rial mesenteric vascular diseases (arterial embolism and non-occlusive) compared to venous thrombosis, ischemic colitis, and other ischemic diseases of the small and large intestine (strangulation, detorsion, etc.). Some studies in the literature have shown that lactate values increase in the 4th h after ischemia in AMI.[25,29] In our study, though the increase in serum lactate values began numerically in the 1st h, it did not reach statistical significance. In the 3rd h lactate was elevated both statistically and numerically and it was observed to increase until the 6th h. Similarly, while the elevation in serum lactate levels was clearly observed in the ischemia groups, the serum thiol values clearly reduced while IMA and serum disulphide values increased as the ischemia duration increased. This situation shows that hypoxia in tissue gradually increased and that serum lactate values increased with the increase in oxidative stress in ischemic tissues and explains the correlation observed between TDH parameters and lactate values.

Our study is the first to investigate TDH parameters in AMI using the method described by Erel and Neselioglu and to assess serum thiol values, disulfide, NT/TT, disulfide/TT, disulfide/NT, IMA, and lactate values together. There are some limitations to our study. The most important limitation is the variation in TDH in all ischemic and hypotensive pathologies, as with serum lactate, IMA, and α-GST.[1,19,27] The most important disadvantage of our study is that just as with parameters such as IMA, serum lactate, and α-GST, TDH will clearly display variability in all diseases causing ischemic, septic, and hypovolemic shock.[30,31] From this aspect, it will be difficult to standardize for patients in clinical practice, because patients with AMI may probably have comorbid ischemic or vascular pathologies like coronary artery disease, peripheral vascular diseases, pulmonary embolism, and deep vein thrombosis. In the name of showing ischemia in the early period in our study, there was a need to compare the efficacy and histopathologically prove the TDH parameters. As a result, two separate arterial ischemia groups were created ending in the 3rd and 6th h. Here, another limitation of the study design is that the sham group was included in all 6 h. However, serum analyses belonging to the sham group resolve this limitation by showing no variation in TDH and other parameters in the 0, 1st, 3rd, and 6th h.

Conclusion

TDH varies in the early period of AMI. The TDH parameters may be used with IMA as diagnostic parameters in patients with suspected AMI in the early period. In addition, TDH may be beneficial to monitor the adequacy of thrombotic, endovascular and surgical treatment given to AMI patients. There is a need for clinical studies in the future to assess TDH in AMI.

Ethics Committee Approval: This study was approved by the Necmettin Erbakan University Animal Experiments Local Ethics Committee (Date: 29.06.2020, Decision No: 2020-030).

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DENEYSEL ÇALIŞMA - *ÖZ*

Akut mezenter iskeminin erken tanısında tiyol disülfit hemostazının rolü: Deneysel çalışma

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AMAÇ: Akut mezenter iskemi (AMİ) nadir görülen ölümcül ve erken tanısı güç olan bir akut karın hastalığıdır. Bu çalışmada amacımız, erken tanısında henüz spesifik biyokimyasal belirteç bulunmayan AMİ'de yeni bir yöntem olan tiyol-disülfit hemostazının (TDH) rolünü, daha önce AMİ'de güvenilirliği ispatlanmış iskemi-modifiye albümin (IMA) ile birlikte değerlendirmektir.
GEREÇ VE YÖNTEM: Çalışmada erişkin 32 adet wistar albino ırkı sıçan kullanılarak dört grup oluşturuldu. Birinci Grup (n=8) kontrol grubu, 2. Grup (n=8) SHAM grubu, 3. Grup (n=8) 3. saat arteryel mezenter iskemi, 4. Grup (n=8) 6. saat arteryel mezenter iskemi grubu olarak belirlendi. 0., 1., 3. ve 6. saatlerde TDH, IMA ve serum laktat değerleri ölçüldü.
BULGULAR: Üçüncü ve 6. saatlerde serum total tiyol, native tiyol (p<.001) değerlerinin belirgin azaldığı, serum disülfit, IMA, laktat (p<.001) değerlerinin belirgin arttığı tespit edildi. Serum tiyol değerleri 1. saatten itibaren azaldığı görülmüştür.
TARTIŞMA: TDH akut mezenter iskemide erken dönemde değişmektedir. TDH parametreleri AMİ şüphesi olan hastalarda erken dönemde İMA ile birlikte tanı parametresi olarak kullanılabilir.
Anahtar sözcükler: Akut mezenter iskemi; disülfit; hemostaz; tiyol.

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