



## Original Contribution

## The value of endoplasmic reticulum stress markers (GRP78 and CHOP) in the diagnosis of acute mesenteric ischemia



Senol Ardic<sup>a</sup>, Aysegul Gumrukcu<sup>a</sup>, Ozgen Gonenc Cekic<sup>a</sup>, Mehmet Erdem<sup>b</sup>, Goksen Derya Reis Kose<sup>c</sup>, Selim Demir<sup>d</sup>, Bestami Kose<sup>a</sup>, Esin Yulug<sup>c</sup>, Ahmet Mentese<sup>e</sup>, Suleyman Turedi<sup>f,\*</sup>

<sup>a</sup> University of Health Science, Faculty of Medicine, Department of Emergency Medicine, Trabzon, Turkey

<sup>b</sup> Karadeniz Technical University, Faculty of Medicine, Department of Medical Biochemistry, Trabzon, Turkey

<sup>c</sup> Karadeniz Technical University, Faculty of Medicine, Department of Histology and Embryology, Trabzon, Turkey

<sup>d</sup> Karadeniz Technical University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Trabzon, Turkey

<sup>e</sup> Karadeniz Technical University, Vocational School of Health Sciences, Program of Medical Laboratory Techniques, Trabzon, Turkey

<sup>f</sup> Karadeniz Technical University, Faculty of Medicine, Department of Emergency Medicine, Trabzon, Turkey

## ARTICLE INFO

## Article history:

Received 20 April 2018

Received in revised form 12 June 2018

Accepted 15 June 2018

## Keywords:

Acute mesenteric ischemia

GRP78

CHOP

Endoplasmic reticulum stress

Oxidative stress

TAS

TOS

OSI

## ABSTRACT

**Aim:** To evaluate levels of the endoplasmic reticulum (ER) stress markers GRP78 and CHOP in acute mesenteric ischemia (AMI) and to examine relations with degrees of AMI-related intestinal injury.

**Materials and methods:** Twenty-four rats were divided into four groups. Group I and Group III represented the control groups, from which blood and tissue specimens were collected 2 and 6 h after laparotomy without superior mesenteric artery (SMA) ligation. Group II and Group IV constituted the ischemia groups, from which blood and tissue specimens were collected 2 and 6 h after SMA ligation. The ER stress markers GRP78 and CHOP, total oxidant status (TOS), total antioxidant status (TAS), and the oxidative stress index (OSI) were investigated in each group. Ileum specimens were assessed in terms of ischemic injury, and appropriate comparisons were performed.

**Results:** Significantly higher GRP78, CHOP, TOS, and TAS values were determined in the ischemia groups (groups II and IV) compared to the control groups (groups I and III). This elevation was greater in the 6 h ischemia group, the group exposed to the greatest ischemic injury (Group IV). Significant and powerful correlation was present between histopathological damage and levels of the ER stress markers and oxidative markers.

**Conclusion:** According to our results, ER stress markers (GRP78 and CHOP) increase significantly following ischemic injury. This elevation has the potential to be used diagnostically and also in prognostic terms due to the powerful correlation it exhibits with AMI-related ischemic injury.

© 2018 Elsevier Inc. All rights reserved.

## 1. Introduction

Acute mesenteric ischemia (AMI) is a potentially fatal vascular emergency in which urgent diagnosis and treatment are essential if mesenteric blood flow is to be restored and intestinal necrosis avoided [1]. Diagnosis relies upon a high degree of clinical suspicion. The primary clinical aim is to prevent the development of ischemia and necrosis-associated complications, such as peritonitis [2]. Signs of peritonitis being observed at physical examination indicate a strong probability of irreversible intestinal ischemia with bowel necrosis [3]. Mortality and morbidity can be significantly lowered by prompt diagnosis before the onset of peritonitis and surgical or endovascular treatment [4]. The identification of a biomarker indicating early

hypoperfusion in AMI before the onset of irreversible intestinal damage would therefore represent a major clinical advance. However, no standardized blood tests or biomarkers capable of screening for AMI in patients presenting with acute abdominal pain are currently available [5, 6]. Contrast computerized tomography (CT) angiography represents the gold standard imaging modality in AMI. CT findings in AMI may be specific or non-specific in character. AMI may still be present even if ischemia-specific CT findings are absent, and non-specific intestinal CT findings may be overlooked at CT examination in the absence of clinical suspicion of AMI [7]. An ideal marker capable of application in the diagnosis of AMI and of guiding these diagnostic algorithms is therefore needed.

The endoplasmic reticulum (ER) is an organelle containing chaperone proteins and enzymes responsible for protein folding. Accumulation of unfolded or misfolded proteins in the ER is a cellular response emerging in case of impairment of ER homeostasis, and is defined as ER stress [8]. Conditions such as ischemia, neurodegenerative diseases

\* Corresponding author at: Karadeniz Technical University, Faculty of Medicine, Department of Emergency Medicine, 61080 Trabzon, Turkey.  
E-mail address: [suleymanturedi@hotmail.com](mailto:suleymanturedi@hotmail.com) (S. Turedi).

and diabetes lead to protein accumulation and toxic effects in the cell in association with ER stress [8, 9]. ER stress has been shown in ischemic cardiac and neurological diseases, neurodegenerative diseases, sepsis, various cancers, inflammatory bowel diseases involving intestinal damage, and in renal diseases. Glucose-regulated protein 78 (GRP78) and C/EBP homologous protein (CHOP) are contemporary, novel biomarkers reflecting ER stress. Studies have concluded that GRP78 and CHOP levels may be useful in diagnosis and prognosis in diseases involving increased ER stress [10–16]. To the best of our knowledge, no previous studies have investigated ER stress in AMI or the diagnostic value of markers showing ER stress.

The purpose of this study was to evaluate levels of the ER stress markers GRP78 and CHOP in an experimentally induced model of AMI and to investigate correlation with degrees of AMI-related intestinal injury. This will be indicative of whether levels of GRP78 and CHOP can be used as biomarkers in the early diagnosis and prognosis of AMI.

## 2. Materials and methods

### 2.1. Study design

This randomized, controlled, non-blinded interventional animal study commenced following receipt of approval from the Karadeniz Technical University Medical Faculty Animal Experiments Ethical Committee.

### 2.2. Setting and population

Twenty-four Sprague-Dawley rats, weighing 350–400 g and aged 3–4 months, bred in the ... Surgical Practice and Research Center, and selected on a random basis were assigned into four groups containing six animals each. The rats were housed in individual cages at  $22 \pm 1$  °C, in a light (12-h dark:light) and humidity controlled environment, with access to water and standard rat chow. Water only was allowed during the final 12 h prior to the study.

### 2.3. Study protocol

The 24 rats in the study were numbered from one to 24 and randomized into six-member groups using the closed envelope method. Group I ( $n = 6$ ) underwent laparotomy only, and was monitored without ligation of the superior mesenteric artery. Blood and tissue specimens were collected after 2 h. In Group II ( $n = 6$ ), experimentally induced mesenteric ischemia was established following laparotomy and ligation of the superior ischemic artery using silk 5/0 surgical suture, and blood and tissue specimens were collected after 2 h. In Group III ( $n = 6$ ), blood and tissue specimens were collected after 6 h, following laparotomy without ligation of the superior ischemic artery. In Group IV ( $n = 6$ ), mesenteric ischemia was induced following laparotomy with ligation of the superior mesenteric artery, and rats were subjected to ischemia for 6 h. Blood and tissue specimens were then collected. The experimental protocol employed was produced from previous experimental AMI studies in the literature [17, 18]. Necrosis in the mucosal villus develops 3–4 h after the onset of ischemia in AMI, and transmural, mural or mucosal infarction develop within 6 h. We therefore selected 2-h and 6-h time points after onset of in order to reflect the early and relatively late periods [2, 19].

General anesthesia was administered to all rats during the experiment with intraperitoneal 50 mg/kg ketamine and 5 mg/kg xylazine. Superior mesenteric artery ligation for induction of mesenteric ischemia was applied to the aortic bifurcation.

### 2.4. Laboratory analysis

Blood specimens were collected from the abdominal aorta. These were then placed into appropriate tubes and centrifuged for 10 min

at  $1800 \times g$ , and the separated sera were used for biochemical measurements.

The ER stress markers GRP78 and CHOP were determined from biochemical measurements. TAS, TOS, and OSI frequently used in previous studies concerning the diagnosis of AMI were also calculated from blood specimens [18, 20].

#### 2.4.1. Determination of GRP78 levels

GRP78 levels in serum specimens were determined using sandwich ELISA kits with two antibodies (SunRed, Catalog No: 201–11-0992, Shanghai, China) in line with the manufacturer's instructions.

#### 2.4.2. Determination of CHOP levels (DDIT3; DNA-Damage-Inducible Transcript 3)

CHOP levels in serum specimens were determined using sandwich ELISA kits with two antibodies (SunRed, Catalog No: 201–11-4608, Shanghai, China) in line with the manufacturer's instructions.

#### 2.4.3. Total Oxidant Status (TOS) determination

TOS values in serum specimens were determined using colorimetric kits (Rel Assay Diagnostics, Catalog No: RL0024, Gaziantep, Turkey) in accordance with the manufacturer's instructions. The results were expressed as  $\mu\text{mol H}_2\text{O}_2$  equivalent/L (micromol  $\text{H}_2\text{O}_2$  equivalent/L).

#### 2.4.4. Total Antioxidant Status (TAS) determination

TAS values in serum specimens were determined using colorimetric kits (Rel Assay Diagnostics, Catalog No: RL0017, Gaziantep, Turkey) in accordance with the manufacturer's instructions. The results were expressed as  $\mu\text{mol trolox}$  equivalent/L (micromol trolox equivalent/L).

#### 2.4.5. Oxidative Stress Index (OSI) calculation

OSI values in serum specimens were calculated using the formula  $\text{OSI} = [(\text{TOS}, \mu\text{mol H}_2\text{O}_2 \text{ equivalent/L}) / (\text{TAS}, \text{mmol trolox equivalent/L})] \times 100$  [21].

### 2.5. Histopathological analysis

The ileum was resected in all rats from all groups. One-centimeter sections were collected for histopathological analysis. Specimens were placed into numbered cassettes containing 10% neutral formaldehyde solution for light microscopic examination, and were fixed in this solution for 48 h. Following dehydration and clearing, they were embedded in paraffin blocks, from which  $5 \mu\text{m}$  ( $\mu\text{m}$ )-thick sections were taken using a fully automatic microtome (Leica RM2255, Tokyo, Japan). Ileum prepares from all groups were stained with hematoxylin and eosin (H&E). Histopathological analysis was performed by an experienced histologist blinded to the study groups. Histopathological examination of prepares was carried out by analyzing five different areas at  $\times 200$  magnification using a light microscope (Olympus, BX51, Japan) with an attached camera (Olympus, DP71, Japan) in the Karadeniz Technical University Medical Faculty Histology and Embryology Department. Each area was scored semi-quantitatively between 0 and 3 in terms of inflammatory cell infiltration, hemorrhage, villous fusion, villous apical surface epithelial degeneration, and vascular congestion; 0, none; 1, mild; 2, moderate, and 3, severe [22].

### 2.6. Statistical analysis

The data obtained in the study were analyzed on SPSS 23 (IBM, SPSS, Armonk, NY, US) and MedCalc (MedCalc Software, Mariakerke, Belgium) software. Compatibility with normal distribution of numerical data in a data set was assessed using the Shapiro-Wilk test. Student's *t*-test was used for two-group comparisons of normally distributed numerical data. ANOVA and the Tukey test for Bonferroni correction were used for multiple group comparisons of normally distributed numerical data. The Mann-Whitney U test was used in two-group comparisons of non-

normally distributed numerical data, while Kruskal Wallis analysis of variance and the Mann-Whitney U test for Bonferroni correction were used in multiple group comparisons. Since biochemical parameters were normally distributed, Pearson's correlation analysis was used to determine relations between these parameters, while Spearman's correlation analysis was used to determine relations with histopathological damage scores, which were not normally distributed. *p* values <0.05 were regarded as statistically significant.

### 3. Results

Biochemical measurements and histopathological damage scores for the various groups in our experimentally-induced AMI model are shown in Tables 1 and 2 and Fig. 1.

Our histopathological examination results show that the experimental AMI model was successfully established. Significantly greater histological damage was observed in ileum tissue in the 2-h and 6-h ischemia groups (groups II and IV) compared to the control groups (groups I and III). This damage was highest in Group IV, which was exposed to AMI for a longer (6 h) period. There was no statistically significant difference between the control groups (groups I and II) in terms of histological damage. Moderate villous fusion and mild inflammatory cell inflammation, villous epithelium degeneration, hemorrhage, and vascular congestion were observed in ileum tissue in Group I. In Group II, we observed moderate inflammatory cell inflammation in ileum tissue and moderate-severe villous fusion, degeneration in the villous epithelium and vascular congestion. In Group III, mild-moderate inflammatory cell inflammation and villous fusion and moderate hemorrhage, degeneration in the villous epithelium and vascular congestion were present in ileum tissue. In Group IV, in addition to severe inflammatory cell inflammation, hemorrhage, villous fusion, and degeneration in the villous epithelium, villous structure was also degenerated in most areas (Fig. 2A–D).

On the basis of our results for GRP78 and CHOP, indicating ER stress, and TOS, TAS and OSI, indicating oxidative stress, all parameters increased significantly with ischemic injury. GRP78, CHOP, TOS, and TAS values were significantly higher in the ischemia groups (groups II and IV) than in the control groups (groups I and III). The highest values were obtained in the 6-h ischemia group (Group IV) in which the highest level of ischemic injury occurred, compared to the 2-h ischemia and control groups. Similarly to the histopathological findings, there was also no difference between the control groups (groups I and III) in terms of biochemical parameters.

Findings for correlation between biochemical parameters and with histopathological damage are shown in Table 3. High positive correlation was present between the ER stress markers GRP78 and CHOP. Moderate levels of correlation were observed with other biomarkers. Significant and powerful correlation was present between both the ER stress indicators GRP78 and CHOP and the oxidative stress indicators TOS and TAS in terms of histopathological damage.

### 4. Discussion

No standardized blood tests or biomarkers capable of application in the diagnosis and prognosis of AMI are currently available. The discovery of a biomarker indicating early hypoperfusion in AMI before the onset of irreversible intestinal damage would therefore represent a significant clinical advance. This precursor study investigated ER stress and levels of ER stress markers (GRP78 and CHOP), together with oxidative stress and levels of oxidative stress markers (TOS, TAS, and OSI) in AMI, in order to determine whether these would be of value in the diagnosis and prognosis of AMI. The results of our study, one of the first to assess ER stress and its markers in blood (GRP78 and CHOP) in AMI, show that ER stress occurs in AMI and increases with the duration of AMI, and that markers reflecting ER stress in blood also increase. This elevation has the potential to be used for diagnostic purposes, and also for prognostic purposes since it exhibits powerful correlation with AMI-related ischemic injury. Similarly, oxidative stress is also involved in the pathophysiology of AMI, and levels of the oxidative stress markers TOS and TAS rise accordingly. This elevation also has the potential for use in the diagnosis and prognosis of AMI.

The ER is an important organelle responsible for the folding and maturation of transmembrane proteins and their transport to the regions where they will perform intracellular functions and for protein quality control during this process. Accumulation of unfolded or misfolded proteins in the ER and the cellular response emerging in the event of impairment of ER homeostasis is known as ER stress. The cell activates unfolded protein response (UPR) as a response to ER stress. The UPR pathway becomes active in order to prevent ER stress and to restore ER homeostasis to its former state [9].

The UPR consists of three different pathways, involving three different ER transmembrane proteins responsible for initiating the response to ER stress: inositol-requiring enzyme 1 (IRE1), protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), and activating transcription factor-6 (ATF6). The chaperone protein BiP (immunoglobulin-binding protein = GRP78: glucose-regulated protein 78) found in

**Table 1**  
Distribution of biochemical parameters by groups.

Biochemical parameters	2 h		6 h	
	Group I	Group II	Group III	Group IV
GRP78 (ng/mL)				
Mean (95% CI)	1.50 <sup>a,c</sup> (0.35–2.66)	3.18 <sup>a</sup> (2.83–3.53)	2.47 <sup>b</sup> (1.96–2.99)	4.09 <sup>b,c</sup> (2.99–5.19)
CHOP (ng/mL)				
Mean (95% CI)	2.55 <sup>a,c</sup> (1.28–3.82)	6.43 <sup>a,d</sup> (5.27–7.58)	2.99 <sup>b,d</sup> (1.90–4.08)	6.17 <sup>b,c</sup> (4.68–7.67)
TOS (μmol H <sub>2</sub> O <sub>2</sub> equivalent/L)				
Mean (95% CI)	3.63 <sup>a,c</sup> (3.31–3.95)	4.82 <sup>a,d</sup> (4.20–5.43)	4.27 <sup>b</sup> (3.92–4.61)	12.31 <sup>b,c,d</sup> (5.53–19.09)
TAS (mmol trolox equivalent/L)				
Mean (95% CI)	0.55 <sup>a,c</sup> (0.35–0.76)	0.78 <sup>a,d</sup> (0.67–0.89)	0.65 <sup>b</sup> (0.54–0.75)	1.45 <sup>b,c,d</sup> (0.95–1.94)
OSI				
Mean (95% CI)	0.70 (0.49–0.91)	0.62 (0.52–0.72)	0.67 (0.54–0.80)	0.81 (0.59–1.02)

a, *p* = 0.005; b, *p* = 0.011; c, *p* < 0.001 for GRP 78;

a, c, d *p* < 0.001; b, *p* = 0.002; for CHOP;

a, *p* = 0.003; b, *p* = 0.028; c, *p* = 0.001; d, *p* = 0.004 for TOS.

a, *p* = 0.038; b, *p* = 0.008; c, *p* < 0.001; d, *p* = 0.001 for TAS;

(Student's *t*-test was used for two-group comparisons, and ANOVA and the Tukey test for Bonferroni correction in multiple group comparisons)

**Table 2**  
Histopathological scores in ileum tissue specimens by groups.

Histological parameters	2 h		6 h	
	Group I	Group II	Group III	Group IV
Inflammation (0–3)				
Median (Minimum-Maximum)	1 <sup>a,c</sup> (1-2)	2 <sup>a</sup> (2-2)	1 <sup>b</sup> (1-2)	3 <sup>b,c</sup> (2-3)
Hemorrhage (0–3)				
Median (Minimum-Maximum)	1 <sup>a,c</sup> (0-1)	2 <sup>a,d</sup> (1-3)	1 <sup>b,d</sup> (0-1)	3 <sup>b,c</sup> (3-3)
Villous fusion (0–3)				
Median (Minimum-Maximum)	1.5 <sup>a</sup> (0-2)	2 <sup>a</sup> (2-3)	1 <sup>b</sup> (1-2)	3 <sup>b</sup> (2-3)
Villous apical surface epithelium degeneration				
Median (Minimum-Maximum)	0.5 <sup>a,c</sup> (0-1)	2 <sup>a,d</sup> (2-3)	0.5 <sup>b,d</sup> (0-1)	3 <sup>b,c</sup> (3-3)
Vascular congestion (0–3)				
Median (Minimum-Maximum)	1 <sup>a,c</sup> (0-1)	3 <sup>a,d</sup> (2-3)	0.5 <sup>b,d</sup> (0-1)	3 <sup>b,c</sup> (3-3)
Total damage score (0–15)				
Median (Minimum-Maximum)	5 <sup>a,c</sup> (4-6)	11.5 <sup>a,d,e</sup> (9-14)	4.5 <sup>b,d</sup> (2-6)	14.5 <sup>b,c,e</sup> (13-15)

a, p = 0.019; b,c p = 0.009 for inflammation;

a,d p = 0.009; b,c p = 0.002 for hemorrhage;

a, p = 0.031; b, p = 0.009 for villous fusion.

a, b,c,d p = 0.002; for villous epithelial degeneration;

a, b,c,d p = 0.002; for vascular congestion;

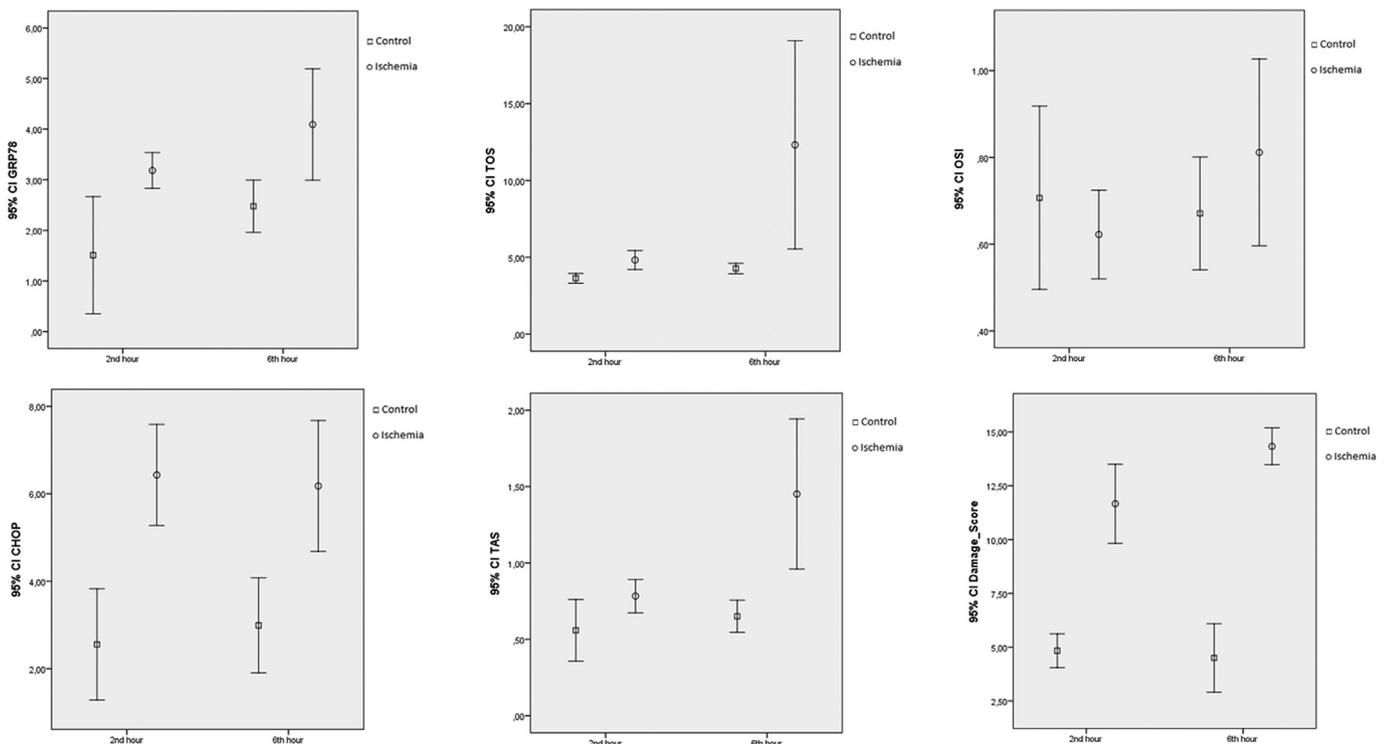
a, b,c,d p = 0.002; e, p = 0.009 for total damage.

(The Mann-Whitney U test was used in two-group comparisons, and Kruskal Wallis analysis of variance and the Mann-Whitney U test for Bonferroni correction in multiple group comparisons)

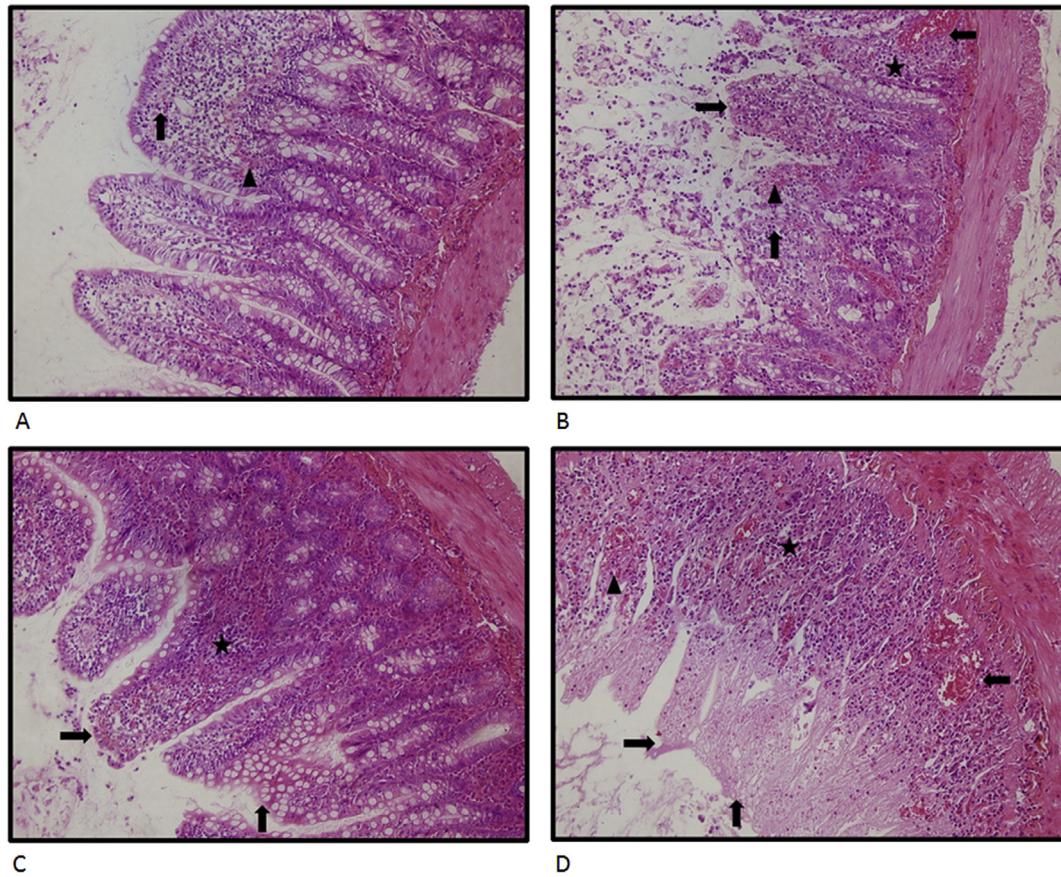
ER is a UPR regulator that plays a key role in IRE1, PERK and ATF6 activation and in the response to ER stress [9, 23]. CHOP transcription is activated by PERK, ATF6 and IRE-1 in the ER membrane. However, the PERK-EIF2a (eukaryotic initiation factor 2)- ATF4 (activating transcription factor-4) pathway is required for CHOP. PERK signaling pathway activation serves to protect cells throughout protein synthesis inhibition in the early ER stress response, and also regulates cell life. However, the PERK-EIF2a-ATF4 pathway is activated by ER stress in the later stage of the ER stress response, inducing CHOP expression and regulating apoptosis [9, 23, 24].

When the cell is at rest, in the absence of ER stress in other words, it is maintained in an inactive state by the ER chaperone GRP78. In the early stages of ER stress, the cell seeks to reduce the accumulation of

misfolded proteins through the UPR, particularly by increasing GRP78 levels, and to support the life of the cell by restoring normal ER functions. However, if ER stress is prolonged, UPR signals intended to protect the cell assume a pro-apoptotic state and conclude with CHOP induction [6, 9, 23]. GRP78 is therefore an ER stress marker released for the purpose of protecting the cell in the early stages of that stress, while CHOP is a marker that regulates controlled cell death-apoptosis as a response to prolonged and continuing ER stress and that may be anticipated to rise later than GRP78 [9]. The present research is a precursor study investigating ER stress in AMI. Considering the importance of early diagnosis in AMI, there is a clear need for a specific marker that rises in the early period and can serve as a guide to early diagnosis and treatment, and that is also associated with the severity or duration



**Fig. 1.** Groups' biochemical parameters and histological damage scores.



**Fig. 2.** Histopathological Injury in Groups' ileum Tissue Specimens. a: ileum tissue section from Group I (H&E X200). Villous fusion (↑), hemorrhage (▲). b: ileum tissue section from Group II (H&E X200). Villous fusion (↑), hemorrhage (▲), inflammatory cell infiltration (star), villous apical facial epithelium degeneration (→), vascular congestion (←). c: ileum tissue section from Group III (H&E X200). Villous fusion (↑), inflammatory cell infiltration (star), villous apical facial epithelium degeneration (→). d: ileum tissue section from Group IV (H&E X200). Villous fusion (↑), hemorrhage (▲), inflammatory cell infiltration (star), villous apical facial epithelium degeneration (→), vascular congestion (←).

of the disease. From that perspective, our study involved analyses performed 2 h after an experimentally induced model of AMI in order to reflect the period in which early diagnosis can be made, and subsequently after 6 h in order to reflect the late period of AMI involving longer exposure to ischemia and therefore greater damage. Our results indicate that significant ER stress occurs in AMI (shown by the levels of the ER stress makers GRP78 and CHOP), and that levels of GRP78 and CHOP in blood increase in line with this stress. GRP78 and CHOP levels in blood were higher in the ischemia groups at both 2 h and at 6 h. Considering the association between GRP78 and CHOP, the first response to ER stress occurs via GRP78, and the levels of this rise first. This increase is a response intended to protect the cell against ER stress. As ER stress persists, in addition to elevation in GRP78, which protects the cell against this stress, levels of CHOP also rise in the context of programmed cell death-apoptosis of cells damaged by ER stress. In later stages of ER stress, the response aimed at protecting cells declines, and GRP78 levels decrease, while CHOP causes apoptosis of these cells by rising

significantly [13, 25]. Our results showed higher levels of both GRP78 and CHOP at 2 h compared to control group levels. This indicates that activity against ER stress arising secondary to ischemia persists at 2 h (due to GRP78 elevation), and that the apoptotic process begins in some cells unable to withstand ER stress (due to CHOP elevation). The same phenomenon was also observed at 6 h. The period prior to 2 h was not evaluated in our study. Nonetheless, considering the relation between GRP78 and CHOP, we think that had blood measurements been performed earlier in AMI (before 2 h), high GRP78 and low CHOP levels would have been observed. Similarly, had blood specimens been collected after 6 h, it might have been possible to observe a decrease in activities against ER stress and significant apoptosis, together with low GRP78 and high CHOP levels.

Very few studies have to date evaluated the relationship between AMI and ER stress. The first was by Bilecova-Rabjdova et al. Those authors applied reperfusion after 1-h mesenteric ischemia and measured CHOP and GRP78 gene mRNA levels 1 h, 24 h, and 30 days after

**Table 3**  
Correlation of degree of histopathologic score, ER stress and oxidative stress markers.

	CHOP <sup>a</sup>		TOS <sup>a</sup>		TAS <sup>a</sup>		OSI <sup>a</sup>		Histopathologic score <sup>b</sup>	
	r	p	r	p	r	p	r	p	r	p
GRP78	0.828	<0.001	0.458	0.025	0.510	0.011	0.224	0.293	0.752	<0.001
CHOP			0.349	0.095	0.469	0.021	-0.025	0.909	0.701	<0.001
TOS					0.930	<0.001	0.603	0.002	0.821	<0.001
TAS							0.297	0.159	0.752	<0.001
OSI									0.165	0.441

<sup>a</sup> According to Pearson correlation analysis.

<sup>b</sup> According to Spearman correlation analysis.

ischemia/reperfusion (I/R). They then compared these with a non-ischemic control group. CHOP gene mRNA levels increased at 1 h in the early post-ischemic period compared to the control group, while GRP78 gene mRNA levels decreased. At 24 h after ischemia, post-I/R CHOP gene mRNA levels decreased compared to the control group, while a very considerable increase was observed in GRP78 gene mRNA levels. At 30 days post-I/R, CHOP gene mRNA levels again increased compared to the control group, while no difference was determined compared to the control group in terms of GRP78 gene mRNA levels [26]. The results of that preliminary studies together with those of subsequent studies were instrumental in new research being undertaken. In another study by the same research group in 2012, ER stress markers were examined at 1 h, 24 h and 30 days following 1-h mesenteric ischemia. The highest GRP78 levels were determined at 1 h after I/R injury, and an accompanying increase in CHOP, or apoptotic signaling, was also observed. GRP78 elevation was highest at 24 h after I/R, while at 30 days, CHOP levels were high and GRP78 levels were low [27]. Although reperfusion was not performed in our study, and only ER stress associated with ischemia was evaluated, an increase in the early period post I/R was observed in both GRP78 and CHOP, in agreement with our own study. This indicated that the increases in GRP78 in the context of early protection against ER stress, and in CHOP, reflecting cell exposure to apoptosis, have the potential for use in the early diagnosis of AMI.

Ischemia alone was investigated in our study. However, the process involved in the two other studies of ER stress in AMI was rather different, since these involved both ischemia and reperfusion. Two different processes may emerge following reperfusion after ischemia. The first is that cells are unable to cope with oxidative stress resulting from reperfusion and consequently with excessive ER stress, and may thus enter into an intensive apoptotic process. A very significant increase in CHOP may therefore be predicted. Another possibility is that under post-reperfusion conditions, the cell is relatively able to cope with and withstand ER stress, and GRP78 increases as a result, while less apoptosis takes place and a partial increase in CHOP may be observed. Since reperfusion was not investigated in our study, we are unable to provide a detailed and reliable analysis on this subject.

This study evaluated TOS, TAS and OSI values in terms of reflecting oxidative stress. When TOS values reflecting the oxidative load and TAS values reflecting the antioxidant response to that load were assessed together, oxidative stress increased (as shown by an increase in TOS) in the second hour after AMI, and antioxidant mechanisms were activated as a response to this (shown by an increase in TAS). This activity reached its highest level in a later stage of ischemia (at 6 h). TOS and TAS values increased together, but oxidative stress predominated (shown by the highest OSI levels). Several studies have investigated TAS, TOS and OSI values in the context of ischemia and reperfusion, and similar findings to our own have been reported [18, 21, 28, 29]. Oxidative stress and ER stress are closely related processes comprising a single entity. The development of oxidative stress and reactive oxygen radicals (ROS) is a complementary component of ER stress, rather than a consequence of it [8]. In support of this idea, we determined powerful positive correlation was determined between TOS and TAS and GRP78 and CHOP levels.

Another striking finding of our study is powerful correlation between AMI-related intestinal injury and the oxidative stress markers TOS and TAS, together with GRP78 and CHOP, markers of ER stress, the complementary component. This finding suggests that all these markers can be used for diagnostic purposes and also have the potential for use in determining prognosis in AMI.

#### 4.1. Limitations

The principal limitation of our study is that it represented experimental research involving rats. Our findings may not therefore exactly reflect the clinical manifestation of AMI in humans. In clinical practice, AMI may be non-occlusive or occlusive, the primary etiology being

identified as mesenteric arterial embolism, mesenteric arterial thrombosis, or mesenteric venous thrombosis [3]. Our research findings are unable to provide any definite information concerning these probable scenarios. In addition, in real life there is a very strong probability in patients with suspected AMI of ER stress and therefore of confounding factors capable of affecting blood ER stress markers. Factors shown to increase ER stress, such as atherosclerosis and smoking will probably affect the biomarkers we evaluated by creating chronic oxidative stress [30–33].

In addition, ER stress markers and oxidative stress markers were measured from blood specimens at 2 h and 6 h after AMI. Necrosis in the mucosal villus develops 3–4 h after the onset of ischemia in AMI, and transmural, mural or mucosal infarction develop within 6 h. We therefore selected 2-h and 6-h time points after onset of in order to reflect the early and relatively late periods [2, 19]. Since there have been no previous studies of blood GRP78 and CHOP levels in AMI, these timings made it impossible to fully resolve some problems. We are unable to provide reliable information concerning the courses of GRP78 and CHOP levels before 2 h or after 6 h. Moreover, our study evaluated only ischemia and associated ER stress, oxidative stress and intestinal injury. This approach was adopted since the purpose of our study was to identify a marker capable of use in the diagnosis of AMI and correlated with the degree of injury. However, the manifestation occurring with treatment after early diagnosis of AMI is I/R injury, not ischemic injury alone. Since post-ischemic reperfusion was not applied in our study, we are unable to offer any evaluation regarding I/R injury. Another limitation is that, in the light of previous studies of ER stress, markers of ER stress were only measured in blood, and no mRNA gene measurement or cellular apoptosis analysis was undertaken. Taking all these limitations into consideration, further studies involving an earlier period after AMI, with a longer monitoring period and more comprehensive analysis, examining I/R injury in addition to ischemic injury and evaluating apoptosis at the cellular level in the light of histopathological findings are now needed.

## 5. Conclusion

In conclusion, oxidative stress and ER stress comprising a single entity both occur in AMI, and the levels of the ER stress markers GRP78 and CHOP in blood increase in association with this. This elevation can be used for diagnostic purposes and also has the potential for use in prognostic terms since it exhibits powerful positive correlation with AMI-related ischemic injury.

### Presented at a meeting

No.

### Grant

No.

### Conflicts of interests statement

The authors declare that they have no significant competing financial, professional or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

### Author contributions

SA, AG, ST, SD, AM: substantial contributions to conception and design, acquisition of data, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content and final approval of the version to be published; ME, GDRS, BK: acquisition of data, interpretation of data, drafting the article or revising it critically for important intellectual content and final approval of the

version to be published; EY: acquisition of data, drafting the article and final approval of the version to be published.

## References

- [1] Oldenburg WA, Lau LL, Rodenberg TJ, Edmonds HJ, Burger CD. Acute mesenteric ischemia: a clinical review. *Arch Intern Med* 2004 May 24;164(10):1054–62.
- [2] Mastoraki A, Mastoraki S, Tziava E, et al. Mesenteric ischemia: pathogenesis and challenging diagnostic and therapeutic modalities. *World J Gastrointest Pathophysiol* 2016;7(1):125–30 [15].
- [3] Bala M, Kashuk J, Moore EE, Kluger Y, Biffl W, Gomes CA, Ben-Ishay O, Rubinstein C, Balogh ZJ, Civil I, Coccolini F, Leppaniemi A, Peitzman A, Ansaloni L, Sugrue M, Sartelli M, Di Saverio S, Fraga GP, Catena F. Acute mesenteric ischemia: guidelines of the World Society of Emergency Surgery. *World J Emerg Surg* 2017 Aug 7;12:38. <https://doi.org/10.1186/s13017-017-0150-5>.
- [4] Tendler DA, Lamont JT. Overview of intestinal ischemia in adults. Collins KA, ed. UpToDate. Waltham, MA: UpToDate Inc. <https://www.uptodate.com/contents/overview-of-intestinal-ischemia-in-adults> (Accessed on March 02, 2018).
- [5] Acosta S, Nilsson T. Current status on plasma biomarkers for acute mesenteric ischemia. *J Thromb Thrombolysis* 2012;33(4):355e61.
- [6] Uzun O, Turkmen S, Eryigit U, Mentese A, Turkyilmaz S, Turedi S, Karahan SC, Gunduz A. Can Intestinal Fatty Acid Binding Protein (I-FABP) be a marker in the diagnosis of abdominal pathology? *Turk J Emerg Med* 2016 Feb 26;14:99–103.
- [7] Karkkainen JS, Acosta S. Acute mesenteric ischemia (part I) Incidence, etiologies, and how to improve early diagnosis. *Best Pract Res Clin Gastroenterol* 2017;31:15–25.
- [8] Bhandary B, Marahatta A, Kim HR, Chae HJ. An involvement of oxidative stress in endoplasmic reticulum stress and its associated diseases. *Int J Mol Sci* 2012;14:434–56.
- [9] Lin JH, Walter P, Yen TSB. Endoplasmic reticulum stress in disease pathogenesis. *Annu Rev Pathol Mech Dis* 2008;3:399–425.
- [10] Qing S, Pengfei R, Yang L, Bo P, Liping D, Ningjing L, Wu Y, Gang Z, Linggen L, Jianying Z. Autoantibodies against glucose-regulated protein 78 as serological diagnostic biomarkers in hepatocellular carcinoma. *Int J Oncol* 2012;41:1061–7.
- [11] Duan SR, Wang JX, Wang J, Xu R, Zhao JK, Wang DS. Ischemia induces endoplasmic reticulum stress and cell apoptosis in human brain. *Neurosci Lett* 2010;475:132–5.
- [12] Khan MM, Yang WL, Wang P. Endoplasmic reticulum stress in Sepsis. *Shock* 2015;44:294–304.
- [13] Guo B, Li Z. Endoplasmic reticulum stress in hepatic steatosis and inflammatory bowel diseases. *Front Genet* 2014;5:1–11.
- [14] Yina XL, Zhanga W, Yanga Y, Shena H. Increasing expression of (CCAAT enhancer binding protein) homologous protein induced by endoplasmic reticulum stress in myocardium after cardiac arrest and resuscitation in rat. *Resuscitation* 2012;83:378–85.
- [15] Abhishek A, Benita S, Kumari M, Ganesan D, Paul E, Sasikumar P, Mahesh A, Yuvaraj S, Ramprasath T, Selvam GS. Molecular analysis of oxalate-induced endoplasmic reticulum stress mediated apoptosis in the pathogenesis of kidney stone disease. *J Physiol Biochem* 2017;73:561–73.
- [16] Zheng YZ, Cao ZG, Hu X, Shao ZM. The endoplasmic reticulum stress markers GRP78 and CHOP predict disease-free survival and responsiveness to chemotherapy in breast cancer. *Breast Cancer Res Treat* 2014;145:349–58.
- [17] Gunduz A, Turedi S, Mentese A, Karahan SC, Hos G, Tatli O, Turan I, Ucar U, Russell RM, Topbas M. Ischemia-modified albumin in the diagnosis of acute mesenteric ischemia: a preliminary study. *Am J Emerg Med* 2008;26:202–5.
- [18] Turkmen S, Mentese S, Mentese A, Sumer AU, Saglam K, Yulug E, Turedi S, Gunduz A. The value of signal peptide-CUB-EGF domain-containing protein 1 and oxidative stress parameters in the diagnosis of acute mesenteric ischemia. *Acad Emerg Med* 2013;20:257–64.
- [19] Sahin A, Altay DA, Demir S, Yulug E, Menteşe A, Tatli O, Karaca Y, Ardic S, Gunduz A, Turedi S. Comparison of the diagnostic values of vascular adhesion protein-1 and intestinal fatty acid-binding protein in the diagnosis of acute mesenteric ischemia. *Eur J Trauma Emerg Surg* 2018 Mar 29. <https://doi.org/10.1007/s00068-018-0944-5>.
- [20] Akcilar R, Akcilar A, Savran B, Ayada C, Koçak C, Kocak FE, Genc O. Effects of ukrain in rats with intestinal ischemia and reperfusion. *J Surg Res* 2015;195:67–73.
- [21] Yulug E, Turedi S, Karaguzel E, Kutlu O, Mentese A, Alver A. The short term effects of resveratrol on ischemia-reperfusion injury in rat testis. *J Pediatr Surg* 2014;49:484–9.
- [22] Gunduz A, Turkmen S, Turedi S, Mentese A, Yulug E, Ulusoy H, Karahan SC, Topbas M. Time-dependent variations in ischemia-modified albumin levels in mesenteric ischemia. *Acad Emerg Med* 2009;16:539–43.
- [23] Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep* 2006;7:880–5.
- [24] Yiming L, Yunshan G, Juan T, Jianli J, Zhinan C. New insights into the roles of CHOP-induced apoptosis in ER stress. *Acta Biochim Biophys Sin* 2014;00:1–12.
- [25] Jian B, Hsieh CH, Chen J, Choudhry M, Bland K, Chaudry I, Raju R. Activation of endoplasmic reticulum stress response following trauma-hemorrhage. *Biochim Biophys Acta* 2008;1782:621–6.
- [26] Bilecová-Rabajdová M, Urban P, Mašlanková J, Veselá J, Mareková M. Analysis of changes in pro (Gadd153) and anti-apoptotic (Grp78) gene expression after ischemic-reperfusion injury of the small intestine. *Prague Med Rep* 2010;111:249–56.
- [27] Urban P, Bilecova-Rabajdova M, Marekova M, Vesela J. Progression of apoptotic signaling from mesenteric ischemia-reperfusion injury to lungs: correlation in the level of ER chaperones expression. *Mol Cell Biochem* 2012;362:133–40.
- [28] El-Gohary OA. Obestatin improves hepatic injury induced by ischemia/reperfusion in rats: role of nitric oxide. *Gen Physiol Biophys* 2017;36:109–15.
- [29] Guclu A, Kocak C, Kocak FE, Akcilar R, Dodurga Y, Akcilar A, Secme M. Micro RNA-320 as a novel potential biomarker in renal ischemia reperfusion. *Ren Fail* 2016;38:1468–75.
- [30] Ivanova EA, Orekhov AN. The role of endoplasmic reticulum stress and unfolded protein response in atherosclerosis. *Int J Mol Sci* 2016 Feb 17;17(2) [pii: E193] <https://doi.org/10.3390/ijms17020193>.
- [31] Hotamisligil GS. Endoplasmic reticulum stress and atherosclerosis. *Nat Med* 2010 Apr;16(4):396–9. <https://doi.org/10.1038/nm0410-396>.
- [32] Tagawa Y, Hiramatsu N, Kasai A, Hayakawa K, Okamura M, Yao J, Kitamura M. Induction of apoptosis by cigarette smoke via ROS-dependent endoplasmic reticulum stress and CCAAT/enhancer-binding protein-homologous protein (CHOP). *Free Radic Biol Med* 2008 Jul 1;45(1):50–9. <https://doi.org/10.1016/j.freeradbiomed.2008.03.003>.
- [33] Kitamura M, Hiramatsu N. The oxidative stress: endoplasmic reticulum stress axis in cadmium toxicity. *Biometals* 2010 Oct;23(5):941–50. <https://doi.org/10.1007/s10534-010-9296-2>.