Diagnostic Value of Procalcitonin Levels in Acute Mesenteric Ischemia

Yunus Karaca[1], Abdulkadir Gündüz[1], Süha Türkmen[1], Ahmet Menteşe[2], Süleyman Türedi[1],
Umut Eryiğit[3], Süleyman Caner Karahan[2]

[1]Department of Emergency Medicine, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey
[2]Department of Biochemistry, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey
[3]Department of Emergency Medicine, Diyarbakır Selahaddin Eyyubi State Hospital, Diyarbakır, Turkey

Background: Acute mesenteric ischemia (AMI) is a potentially fatal disease. Difficulties in diagnosis make it essential to find early biomarkers.

Aims: This study investigated the diagnostic value of procalcitonin (PCT) levels in AMI.

Study Design: Animal experimentation.

Methods: Rats were divided into six groups of six animals each. In the experimental group, an experimental ischemia model was established by clamping the superior mesenteric artery from the aortic outflow tract. Blood and tissue specimens were collected from rats in the experimental mesenteric ischemia model at 30 min and 2 and 6 h, and these were compared with specimens from the respective control groups. PCT levels were compared at 30 min and 2 and 6 h.

Results: PCT levels were 185.3 pg/mL in the control group and 219.3 pg/mL in the study group, 199.6 pg/mL in the control group and 243.9 pg/mL in the study group, and 201.9 pg/mL in the control group and 286.9 pg/mL in the study group, respectively, at 30 minute, 2 and 6 hours. Significant differences were determined between 6-h control group and ischemia group PCT levels (p=0.005).

Conclusion: The absence of a significant increase in PCT levels in the early period, while a significant difference was detected in the later period (6 h), shows that PCT levels rise late in mesenteric ischemia and can be a marker in the late period.

Keywords: Mesenteric ischemia, procalcitonin, intestinal ischemia

Acute mesenteric ischemia (AMI) is a rare condition, but one that frequently involves high mortality (60%–80%) (1). Although it has different etiological causes, common outcomes of mesenteric ischemia are intestinal necrosis and gangrene (2). The most important factor determining mortality is early diagnosis and early surgical intervention. Laboratory findings in the early period are non-specific. Biomarkers to serve as guides in diagnosis are therefore needed (3).

Abdominal sepsis develops following inflammation arising from post-mesenteric ischemia and subsequent bacterial translocation. Procalcitonin (PCT) is a molecule consisting of 116 amino acids and with a molecular weight of 12793 Daltons. It plays a proinflammatory cytokine-like role and its levels rise in severe bacterial, fungal and parasitical infections, autoimmune diseases, sepsis and multiorgan failure (4). It is a biomarker used in the diagnosis of sepsis in the early period (5). PCT has been shown to begin to rise in the first 4 h, reach a peak in 6–8 h and remain high for 24 h (6,7).

This experimental study was intended to evaluate changes in PCT levels from intestinal segments exposed to different periods of ischemia in an experimentally induced ischemia model. It was also intended to determine the PCT levels in mesenteric ischemia and to examine the relations between PCT levels and lactate and histological scores.

MATERIALS AND METHODS

The study was initiated once ethical committee approval had been obtained. Thirty-six Sprague Dawley rats weighing 250–300 g were randomly divided into six groups of six animals each.

The standard experimental animals were kept under laboratory conditions. Following a 12 h fasting period, general anesthesia was performed with intramuscular administration of 50 mg/kg ketamine and 5 mg/kg xylazine. All rats were cannulated with a 24 G venous cannula from the left femoral vein and infused with 4 mL/kg saline. Mesenteric ischemia was established in the study group by clamping the superior mesenteric arteries (SMAs) from the aortic outflow site. Blood and tissue specimens were collected after 30 min, 2 and 6 hours. Laparotomy alone was performed on the rats in the control groups, and blood and tissue specimens were collected at the same intervals and then examined. Blood specimens were taken from the abdominal aorta and placed into tubes containing citrate. Tubes were centrifuged for 15 min at 1000 rpm, and plasma PCT and lactate levels in the separated sera were measured. PCT and lactate levels were determined for each group and compared using macro- and microscopic tissue examination.

Ileum segments 1 cm in length were taken from all study groups and examined histopathologically in order to determine ischemia-related changes. Injury in the preparations obtained was evaluated under a light microscope by a histologist blinded to the study groups.

Procalcitonin levels were determined using enzyme-linked immunosorbent assay (ELISA) kits (USCN Life Science Inc. Wurhan, P.R. China) following the manufacturer's recommendations. Specimen absorbances were measured using a VERSA max tunable microplate reader (Designed by Molecular Devices in California, USA) at a wavelength of 450 nm. Lactate levels were measured using a Roche Vitros autoanalyzer at the Karadeniz Technical University Faculty of Medicine's Farabi Hospital Biochemistry Laboratory.

Statistical analysis
The control laparotomy groups and ischemia groups were analyzed separately. SPSS 13 for Windows (SPSS Inc., Chicago, IL, USA) was used to analyse all data obtained in the study.

These groups' PCT and lactate levels were investigated using Kruskal Wallis analysis of variance (Bonferroni corrected Mann-Whitney U test). All ischemia groups were compared with their corresponding control groups in terms of the same parameters using the Mann-Whitney U test. $p<0.05$ was regarded as statistically significant.

RESULTS

Biochemical analyses
Mean biochemical values determined in all groups in the mesenteric ischemia model established are shown in Table 1. When biochemical parameters were compared between the ischemia and control groups for the same periods, significant differences in lactate levels were only determined between the 2-h control and ischemia groups and in PCT levels between the 6-h ischemia and control groups ($p=0.017$ for lactate; $p=0.005$ for PCT). No significant difference was determined in other comparisons ($p>0.05$ for all periods and comparisons).

Time-dependent changes in biochemical parameters calculated from intra-group analysis of the ischemia and control groups were not significant ($p>0.05$ for all parameters).

Histopathological evaluation
According to the experimental protocol, once blood specimens had been collected at the end of the specified intervals, the rats were sacrificed and intestinal/ileum tissues evaluated under light microscopy. Upon light microscopic examination, various changes increasing over time were observed in the mucosa and submucosa in the control groups administered laparotomy alone (Figure 1a-c). Figure 1a shows a normal histological architecture, Figure 1b shows mild leukocyte infiltration and Figure 1c reveals a leukocyte and occasional villus conglomeration.

Increasing time-dependent changes were observed in the ischemia groups in which SMA occlusion was established. Mild leukocyte infiltration, villus conglomeration and hemorrhage within villi were observed in the 30-min group (Figure 2a). Widespread leukocyte infiltration, hemorrhage and villus degeneration were present in the 2- and 6-h groups (Figure 2b, c).

TABLE 1. Procalcitonin and lactate values compared by group

Time	30 min		2 h		6 h	
Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Procalcitonin (pg/mL)	185.3	219.3	199.6	243.9	201.9	286.9*
Lactate (mg/dL)	12.2	13.7	10.3	19.1*	20.1	20.4

* p<0.05

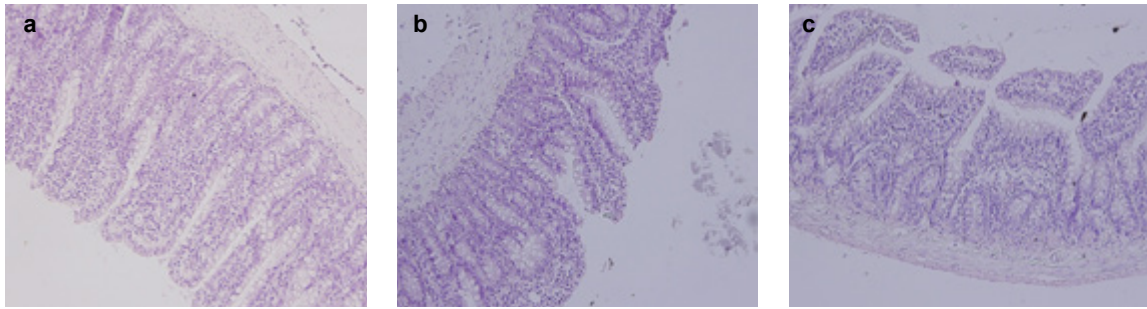Karaca et al. Procalcitonin Levels in Acute Mesenteric Ischemia

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FIG. 1. a-c. A normal histological architecture (a), mild leukocyte infiltration (b), a leukocyte and occasional villus conglomeration (c) (HE x 200)
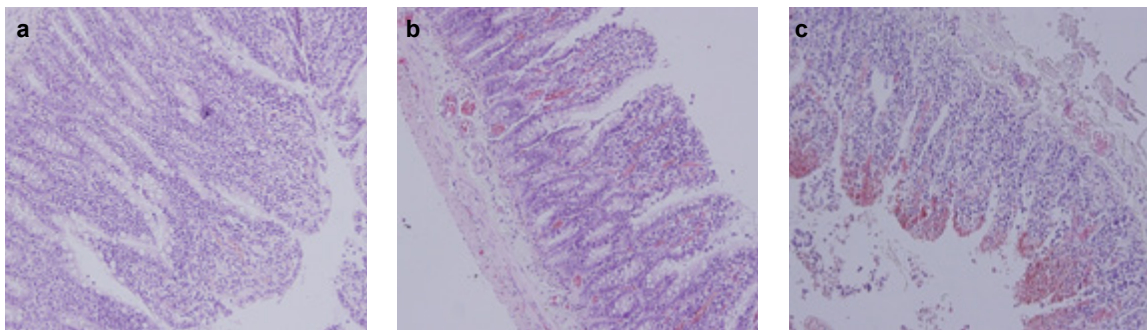

FIG. 2. a-c. Mild leukocyte infiltration, villus conglomeration and hemorrhage within villi in the 30-min group (a), widespread leukocyte infiltration, hemorrhage and villus degeneration in the 2-h and 6-h groups (b, c) (HE x 200)

Histopathological damage scores determined in the groups are shown in Table 2. Comparison of histopathological damage between the control and ischemia groups for the same time periods revealed significantly greater injury in the ischemic groups (p=0.009 for 30 min, p=0.04 for 2 h, p=0.001 for 6 h).

Results of the analysis of correlations of the parameters evaluated in this study are shown in Table 3. When the correlation between parameters was examined, only lactate levels and histopathological damage scores were significantly positively correlated.

DISCUSSION

Various biochemical markers have been investigated for the diagnosis of AMI, but no sufficiently reliable marker has yet been identified. Several parameters have been studied for diagnosis of AMI, such as whole blood count and white cell counts, organic-inorganic phosphate, lactate dehydrogenase (LDH), amylase, acid phosphatase, alkaline phosphatase, creatinine phosphokinase, lactate, alanine transaminase (ALT), aspartate transaminase (AST), D-dimer, peritoneal pH measurement, nitric oxide, blood and peritoneal potassium levels, lipase and intestinal fatty acid binding protein (I-FABP), but none have entered into clinical use (9). D-lactate is a product of bacterial fermentation. It is produced by microflora in the ischemic intes-

tinal flora and enters the circulation in association with impairment of the mucosal barrier. In one study by Lange et al. (10), lactate was 100% sensitive as a marker for AMI, but only 42% specific. In an experimental study by Kulaçoğlu et al. (11), a significant increase in plasma lactate levels was determined in the mesenteric ischemia induction group. Another study concluded that serum lactate measurement was useful for diagnostic purposes but could only be confirmatory (12). In our study, lactate levels only rose in a statistically significant manner between the 2-h mesenteric ischemia group and the corresponding control group. This finding is compatible with data from the literature suggesting that lactate levels can be used in the diagnosis of AMI, particularly in the early periods.

D-dimer is a marker that has been much investigated in recent years. Several studies have shown that D-dimer may be an effective parameter in the diagnosis of AMI. One study in particular, by Acosta et al. (13), reported D-dimer sensitivity of 100% and specificity of 36%. Experimental studies on rats by Altınyollar and Kurt showed that d-dimer levels rise following ischemia (14,15).

In a recent experimental study involving AMI, Rau et al. (16) compared MDA, lactate and ischemia modified albumin levels and determined that variables measured at 30 min and 2 and 6 h were higher in the ischemia group compared to the control group for all parameters. We determined a similar finding for 2-h lactate levels in our study as this study.

TABLE 2. Histopathological damage scores of groups

	Time					
	30 minute		2 hour		6 hour	
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Total Histopathological Damage Score	1.5±1.36	6.0±2.50	3.5±1.50	9.0±3.84	5.0±1.57	12.0±1.03

* $p < 0.05$

TABLE 3. Correlation of biochemical and histopathological score parameters

	Lactate		Histopathological Damage Score	
	r	p	r	p
Procalcitonin	-0.136	0.430	0.285	0.092
Lactate			0.509	0.002

Procalcitonin is a novel biomarker that is used for infectious conditions. It plays a proinflammatory cytokine-like role and its levels rise in severe bacterial, fungal and parasitical infections, autoimmune diseases, sepsis and multiorgan failure (4) It has been found that PCT levels rise earlier than C-reactive protein (CRP) and white blood cells in sepsis and infectious diseases (17). PCT levels can be detectable after 2–3 hours of infection, increase rapidly in 6–8 hours and reach a peak value in 12 hours (18). Several studies have shown that PCT can be used in early and differential diagnosis of bacterial sepsis, and a sensitivity of 60%–100% and specificity of 79%–100% have been reported. Güven et al. (19) reported that PCT, found to be more specific than C-reactive protein in the diagnosis of bacterial sepsis, can be used in the early diagnosis of bacterial infections in emergency departments. Steinwald et al. (20) reported that PCT levels can be used as a marker for the degree of sepsis and mortality. In a study investigating whether PCT levels can be used in the early diagnosis of AMI in an experimental AMI model, Karabulut et al. (21) reported that PCT levels rise in the early period in AMI and can be used as a diagnostic and prognostic marker.

In conclusion, our study is one of the first in the literature to evaluate PCT levels in AMI, and is therefore original. Bearing in mind the bacterial translocation and tendency to sepsis that take place in AMI patients, although PCT levels rise in AMI patients and we think that this elevation can be diagnostically useful, PCT levels have been proven to rise significantly only in the late periods of experimentally induced mesenteric ischemia. The absence of any significant increase in the first 2 h, regarded as a critical period for early diagnosis, suggests that PCT levels are not suitable for use in early diagnosis. Significant differences in the later period (6th h) shows that PCT levels rise in the late period in mesenteric ischemia and can be a marker in the late period.

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