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ORIGINAL ARTICLE

Comparison of the diagnostic values of vascular adhesion protein-1 and intestinal fatty acid-binding protein in the diagnosis of acute mesenteric ischemia

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Abstract

Objectives The aim of this study is to compare the diagnostic values of plasma levels of vascular adhesion protein-1 (VAP-1) and intestinal fatty acid-binding protein (I-FABP) for diagnosing acute mesenteric ischemia (AMI).

Methods The study used a randomized, controlled experimental design. Forty-two female Sprague–Dawley rats were divided into three control groups and three ischemia groups. Plasma VAP-1 and I-FABP levels were measured, and the extent of ischemic damage was determined using a histopathological damage score in terminal ileum tissue samples.

Results In the early phase of AMI (i.e. at the 30-min time point), VAP-1 levels did not differ between the control and ischemia groups ($p > 0.05$), but I-FABP levels were significantly higher in the ischaemia groups ($p = 0.017$). Although both VAP-1 and I-FABP levels increased in the ischaemia groups, only VAP-1 levels showed a significant increase compared to the control group at the 2-h time point ($p = 0.011$). Ischemic damages associated with AMI became the most prominent at the 6-h time point. During this phase, both VAP-1 and I-FABP levels were significantly higher in the ischemia groups than in the control groups ($p = 0.007$ and $p = 0.002$, respectively). Both VAP-1 and I-FABP levels showed a significant correlation with ischemic changes, but a higher correlation was observed for VAP-1 levels ($r = 0.771$).

Conclusions Both I-FABP and VAP-1 levels were useful for diagnosing AMI, but VAP-1 levels correlated better with the extent of ischaemic damage.

Keywords Acute Mesenteric Ischemia · VAP-1 · I-FABP

Introduction

Acute mesenteric ischaemia (AMI) is a medical emergency with a mortality rate of 60–80%. In clinical practice, AMI is divided into four pathological groups: (1) acute mesenteric arterial embolism, (2) acute mesenteric arterial thrombosis, (3) non-occlusive mesenteric ischaemia and (4) mesenteric venous thrombosis. Acute mesenteric arterial embolism constitutes approximately 50% AMI cases, whereas acute mesenteric arterial thrombosis has the second highest prevalence of 25% [1, 2]. Because the primary clinical objective is to prevent the complications associated especially with acute ischaemia and necrosis, diagnosis within the first 6–8 h is extremely important for decreasing mortality [2]. Considering that the symptoms and laboratory findings in AMI are nonspecific, diagnosis may be delayed or even overlooked. The pathophysiological mechanisms underlying high mortality rates are

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intestinal necrosis associated with delayed diagnosis and the systemic inflammatory response syndrome occurring as a result of intestinal perforation [3].

There are no specific laboratory biomarkers for diagnosing AMI. The diagnosis is made by performing spiral computed tomography (CT) angiography in cases with a high index of suspicion, and this method has a high specificity and sensitivity [4]. However, reliable biomarkers with high specificity and sensitivity are needed to decrease mortality by allowing for an early diagnosis and avoiding the unnecessary use of advanced imaging methods (e.g. spiral CT angiography) with high costs or high risk of complications (e.g. contrast nephropathy).

Many studies on AMI diagnosis have reported biomarkers, such as intestinal fatty acid-binding protein (I-FABP), glutathione-s-transferase in the mucosa and small intestine and D-lactate produced by small intestinal bacteria such as *Escherichia coli* [5, 6]. I-FABP is an intracellular protein whose levels to increase in conditions such as inflammation and ischaemia [6]. I-FABP levels are low in the plasma of healthy individuals, whereas I-FABP levels increase in the plasma shortly after AMI and inflammatory episodes. Because of its low molecular weight, I-FABP in the systemic circulation is filtered by the glomeruli and excreted from the kidneys, and it can be easily detected in plasma. Clinical studies have shown that I-FABP levels may increase in intestinal ischaemia and that it is a sensitive marker [7, 8]. Although, I-FABP is known as a clinical biomarker for intestinal ischemia and mesenteric infarction, it is not in use at the moment in daily clinical practice, but still utilized in experimental studies.

Vascular adhesion protein-1 (VAP-1) is an endothelial sialoglycoprotein found on the surfaces of adipocytes, endothelial cells and smooth muscle cells, and it is released during the inflammatory processes [9–11]. VAP-1 is a cell adhesion molecule that plays a role in atherosclerosis and inflammation. Therefore, it is released from the endothelium for the purpose of inducing inflammation and neutrophil migration. Contrary to other adhesion molecules, it is argued that VAP-1, which also functions as an enzyme (semi-carbazide-sensitive amine oxidases), may be used as a potential biomarker in the early phase of ischaemic vasculopathy because it is released into systemic circulation, particularly in ischaemic events [12]. To our knowledge, no studies have evaluated whether these potential biomarkers could be used for diagnosing AMI. The present study is designed on the basis of the hypothesis that VAP-1 levels may increase in AMI patients and that increased levels of it could be used for diagnostic purposes.

The aim of the study is to investigate the time-dependent changes in VAP-1 levels following mesenteric ischaemia, and to analyze its potential use as a biomarker for AMI by comparing it with I-FABP levels, which have been reported to be useful as a diagnostic marker in previous clinical studies.

Materials and methods

Study design

This was a randomized, controlled, nonblinded interventional animal study. Approval for the experimental protocol was obtained from Institutional Animal Care and Ethics Committee (2016/24).

Study setting and population

Fourty-two female Sprague–Dawley rats (10 weeks old and weighing approximately 240–280 g) were used. The rats were kept in steel cages until the day of the study at room temperature (22 °C) and were given water and standard rat chow. For the last 12 h before the study they were given only water.

Study protocol

The rats were randomized into six groups of seven animals each. During allocation into randomized groups, the 42 rats were numbered from 1 to 42. All of these numbers were printed out and put by one into opaque envelopes and groups were established by a blinded person drawing of the envelopes [13]. The rats were randomly allocated into groups one by one. To sustain uniformity through the study, interventions were conducted simultaneously on each rat in each group. At the begining of the study protocol, general anesthesia was administered with the intramuscular injection of 50 mg/kg ketamine and 5 mg/kg xylazine. After the general anesthesia, all rats were cannulated from the left femoral vein and infused with saline at 4 mL/kg/h. Respiratory rate, oxygen saturation, and body temperatures were continuously monitored.

The control groups (Groups 1, 3 and 5) underwent a simple laparotomy, and blood and tissue samples were taken at 30 min, 2 and 6 h thereafter, respectively. The superior mesenteric artery (SMA) was ligated in rats in the ischemia groups (Groups 2, 4 and 6), and blood and tissue samples were taken at 30 min, 2 and 6 h thereafter, respectively. 4-cm incisions were made for laparotomy and the SMA was ligated with 3–0 silk at the aortic bifurcation in the ischemia groups (Groups 2, 4, and 6). Blood samples were taken from aorta and specimens for histopathologic examination were taken with relaparotomy after thirty minutes in Groups 1 and 2, after two hours in Groups 3 and 4, and after six hours in Groups 5 and 6. This model was derived from the available literature [14–17].

Laboratory analysis

Approximately 3 ml blood samples of aortic blood was taken from each rat, placed into citrated tubes, and centrifuged for 10 min at 3000 rpm. Plasma VAP-1 and I-FABP levels were measured and compared by a biochemist blinded to the study groups.

Vap-1 and I-FABP measurement

An enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, Catalogu No. E-EL-R1209, Wuhan, P.R. China) was used to determine plasma VAP-1 levels and an ELISA kit (Elabscience, Catalog No. E-EL-R0572, Wuhan, P.R. China) was used to determine plasma I-FABP levels following the manufacturer's instructions. All specimen absorbances were determined on a VERSA max tunable microplate reader (Molecular Devices, Sunnyvale, CA) at a wavelength of 450 nm. We created a standard curve using absorbance values against standard concentrations. VAP-1 levels in the samples were measured using the standard curve. Results were expressed as ng/mL.

Determination of intestinal damage

A 1-cm-long section of ileum tissue was taken from each group and examined both macroscopically and microscopically. Tissues were fixed for 48 h in 10% formaldehyde solution. After being fixed, they were dehydrated by being passed through 80, 90, 96, and 100% denatured alcohol series. After being rendered transparent in xylene they were fixed in paraffin. Sections of 4-µm thickness were taken from the paraffin blocks using a fully automatic microtome (RM 2255, Leica, Tokyo, Japan). The sections were then stained with hematoxylin and eosin (H&E). Histologic examination of the preparates was performed under a light microscope by an experienced histologist blinded to the study groups. All preparates were examined for general morphology under a light microscope (BX 51, Olympus, Tokyo, Japan) under magnifications of X100. Five different regions, at a magnification of X200 were evaluated in each preparate. Each region was scored semiquantitatively from 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe) in terms of inflammatory cell infiltration, haemorrhage, villous fusion, and epithelial degeneration in the apical regions of villi [15]. The avarage scores for five regions for each parameter was determined and the total histopathological injury score (THDS) was calculated using the avarage scores and statistically compared between the groups. The highest possible score is, therefore, 12. The intestinal mucosa and submucosa were assessed in this histopathological examination. AMI-related intestinal mucosal necrosis was evaluated.

Data analysis

Statistical analysis was performed using SPSS 23.0 (IBM SPSS, Armonk, NY) and MedCalc 17.2 (MedCalc Software, Mariakerke, Belgium) statistical software. Analysis of the plasma VAP-1 and I-FABP levels of the same interval control and ischemia groups was performed using the Mann–Whitney *U* test. Time-dependent changes in parameters were analyzed using Kruskal–Wallis analysis of variance (Mann–Whitney *U* test with corrected Bonferroni test). Spearman's correlation analysis was used to assess the relationship between biochemical parameters and histopathologic scores. Statistical significance was set at $p < 0.05$. Post hoc power analysis has been performed using G*Power 3.1 statistical software.

Results

Time-dependent changes in plasma VAP-1 levels, plasma I-FABP levels and the results of histopathological examinations in the groups are provided in Fig. 1.

Histopathological damage

The results of histopathological examinations in the groups are shown in Table 1. Histopathological damage, assessed using the total score of histopathological damage, was significantly more prominent in the ischaemia groups (Group 2, 4, 6) than in the control groups (Group 1, 3, 5) at 30 min, 2 h, and 6 h (Groups 1 & 2, $p = 0.002$; Groups 3 & 4, $p = 0.012$; Groups 5 & 6, $p = 0.002$) (Fig. 2).

When the control groups were evaluated, compared to the finding at the 30-min time point, the increase in ischaemic damage was statistically significant at the 2- and 6-h time points, even though only a simple laparotomy was performed (Groups 1 & 3 and Groups 1 & 5, $p = 0.001$); however, the levels of damage at 2 and 6 h were similar (Groups 3 & 5, $p = 0.259$).

When the ischaemia groups were evaluated, there was increasing ischaemic damage over time (Groups 2 & 4, $p = 0.023$; Groups 2 & 6, $p = 0.002$; Groups 4 & 6, $p = 0.007$).

Biochemical results

The biochemical results of the groups are shown in Table 2. When the findings within control groups and ischaemia groups were compared at the 30-min, 2-h and 6-h time points, there was a significant increase in VAP-1 and I-FABP

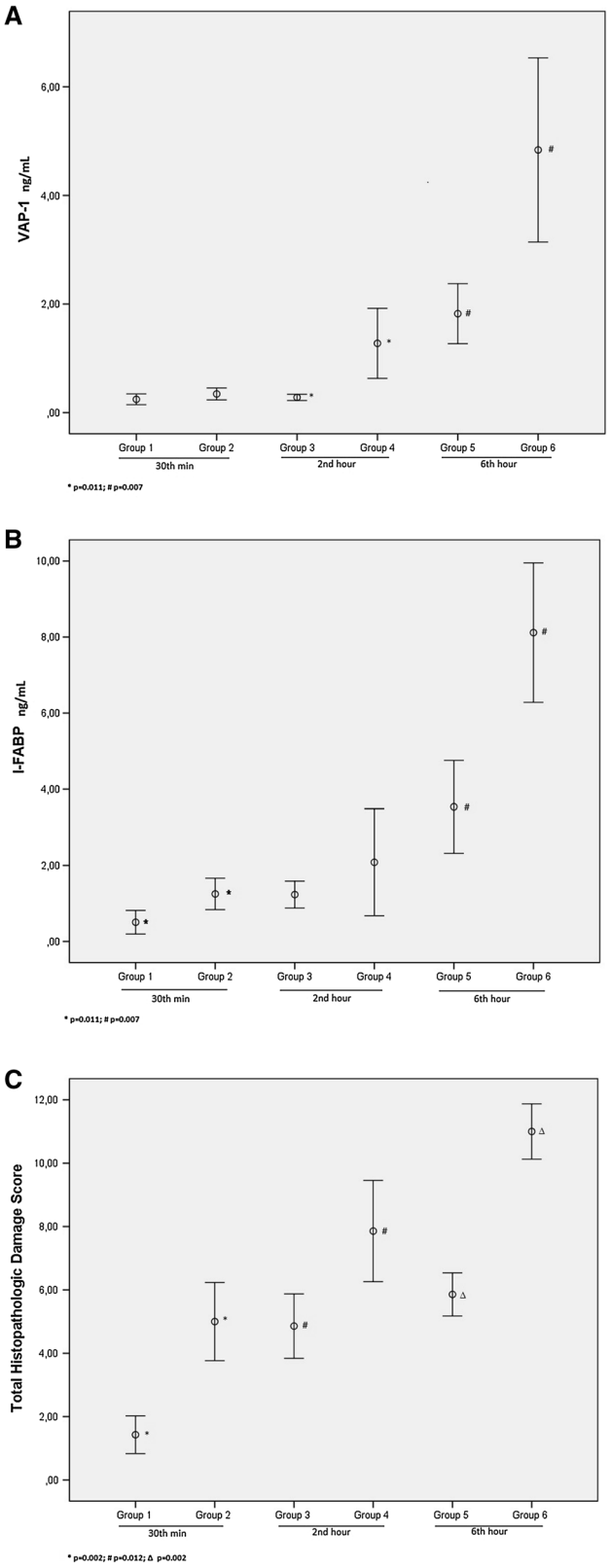

Fig. 1 a, b, c Time-dependent changes in plasma VAP-1 levels, I-FABP levels and total histopathological damage scores among the groups

levels in the ischaemia groups. At the 30-min time point, the increase in I-FABP levels was statistically significant ($p=0.017$ for I-FABP and $p=0.128$ for VAP-1). At the 2-h time point, a similar trend was noted for VAP-1 levels, and levels were significantly higher in the ischaemia group than in the control group ($p=0.011$ for VAP-1 and $p=0.710$ for I-FABP). At the 6-h time point, both VAP-1 and I-FABP levels were significantly higher in the ischaemia group than in the control group ($p=0.007$ for VAP-1 and $p=0.002$ for I-FABP).

When the control groups were evaluated, even though only simple laparotomy was performed, the increase in I-FABP and VAP-1 levels were statistically significant at the 6-h time point, compared to the 30-min and 2-h time points (Groups 1 & 5, Groups 3 & 5, $p=0.002$).

When the ischaemia groups were evaluated, there was a significant increase in I-FABP and VAP-1 levels, similar to the increasing ischaemic damage over time following AMI (Groups 2 & 4, $p=0.018$ for VAP-1, $p=0.565$ for I-FABP; Groups 2 & 6, $p=0.002$ for VAP-1 and I-FABP; Groups 4 & 6, $p=0.006$ for VAP-1, $p=0.003$ for I-FABP).

Correlation between histopathological damage and biochemical parameters

According to the results of Spearman's correlation analysis, there is a positive correlation between VAP-1 and I-FABP levels ($r=0.761$, $p<0.001$). And, the correlation between histopathological damage associated with AMI and both marker levels were statistically significant, but the correlation was higher for VAP-1 levels ($r=0.771$ for VAP-1 and $r=0.615$ for I-FABP, for both $p<0.001$).

Power analysis result

Post-hoc power analysis has been performed based on VAP-1 levels. The primary outcome of the difference between the control and experimental groups at 2 h was evaluated as significant, and the power of our study using the existing numbers of animals was assessed at 80%.

Discussion

In the present study, time-dependent changes in plasma VAP-1 levels as a potential ischaemic marker after AMI were demonstrated in an experimental rat model. In addition, the correlation of these levels with histopathological damage was analysed, and the value of VAP-1 levels and FABP-1 levels for diagnosing AMI were compared, because the FABP-1 levels have been reported as another potential biomarker for diagnosing AMI. In our study, both FABP-1 and VAP-1 levels increased in correlation with ischaemic

Table 1 Time-dependent changes in plasma VAP-1 and I-FABP levels and the comparison between groups

	Time					
	30 min		2 h		6 h	
	Group 1 Control	Group 2 Ischemia	Group 3 Control	Group 4 Ischemia	Group 5 Control	Group 6 Ischemia
Inflamatory cell infiltration*						
Median	0	1	1[a]	2[a]	1[b]	3[b]
IQR	0–1	1–1	1–1	2–2	1–2	2–3
Villus fusion						
Median	1	1	2	2	2	3
IQR	0–1	1–2	2–2	1–2	2–3	2–3
Villus degeneration[¥]						
Median	0[a]	2[a]	1[b]	2[b]	1[c]	3[c]
IQR	0–0	1–2	0–2	2–3	1–1	3–3
Villus haemorrhage[#]						
Median	0[a]	1[a]	1[b]	2[b]	1[c]	3[c]
IQR	0–1	1–2	1–1	2–3	1–1	3–3
Total histopathologic damage Score[¶]						
Median	1[a,d,e]	5[a,f,h]	5[b,d]	8[b,h,g]	6[c,e]	11[c,f,g]
IQR	1–2	3–7	3–6	7–10	5–7	10–12

*a, b: Comparisons between Control and Ischemia groups according to Mann–Whitney *U* Test, $p \leq 0.05$ is statistically significant. a (Groups 3&4), $p = 0.002$; b (Groups 5&6), $p = 0.004$

[¥]a, b, c : Comparisons between Control and Ischemia groups according to Mann–Whitney *U* Test, $p \leq 0.05$ is statistically significant. a (Groups 1&2), $p = 0.007$; b (Groups 3 &4), $p = 0.017$; c (Groups 5&6), $p = 0.000$

[#]a, b, c : Comparisons between Control and Ischemia groups according to Mann–Whitney *U* Test, $p \leq 0.05$ is statistically significant. a (Groups 1&2), $p = 0.026$; b (Groups 3&4), $p = 0.016$; c (Groups 5&6), $p = 0.001$

[¶]a, b, c : Comparisons between Control and Ischemia groups according to Mann–Whitney *U* Test, $p \leq 0.05$ is statistically significant. a (Groups 1&2), $p = 0.002$; b (Groups 3& 4), $p = 0.012$; c (Groups 5&6), $p = 0.002$. d, e: Comparisons in Control groups (Group 1,3,5) according to Mann–Whitney *U* Test with Bonferroni correction, $p < 0.016$ is statistically significant; d (Groups 1&3), $p = 0.001$; e (Groups 1&5) $p = 0.001$. f, g: Comparisons in Ischemia groups (Groups 2,4,6), according to Mann–Whitney *U* Test with Bonferroni correction, $p < 0.016$ is statistically significant. f (Groups 2&6), $p = 0.002$; g (Groups 4&6) $p = 0.007$; h (Groups 2&4), $p = 0.023$

damage, and both were shown to be useful biomarkers for diagnosing AMI. The followings were additional deductions from the findings: FABP-1 levels were more sensitive in the early 30-min period after AMI than VAP-1 levels; VAP-1 levels increased and were more sensitive at 2-h time point compared to I-FABP levels; and both I-FABP and VAP-1 levels were useful for diagnosing AMI at the 6-h time points. However VAP-1 levels better correlated with the ischaemic damage.

In the present study, at the 30-min time point, there was a significant histopathological damage in the intestines, despite the short duration of ischaemia. The levels of both biomarkers that we evaluated increased in the ischaemia group compared to the control group; however, only the increase in I-FABP levels was statistically significant. The finding suggests that in the early phase of AMI, I-FABP levels are a more useful indicator of ischaemia, and that VAP-1 levels are not diagnostic until after this phase. The reason for the early increase may be because I-FABP is a cytosolic protein present in mature intestinal cells, and it is rapidly released into the systemic circulation after ischaemic damage because of its low molecular weight (14–15 kDa) [18, 19]. On the other hand, high molecular weight of VAP-1 (170–180 kDa) may delay its release into the systemic circulation, resulting in a delayed increase in plasma levels [20]. Thus, evaluating FABP-1 levels may be more advantageous than VAP-1 levels in the early phase of AMI. Nevertheless, despite its advantage, patients usually do not have clinical manifestations in the first 30 min after AMI, and their admission to the hospital may be delayed. Moreover, diagnosis in the early phase may be missed because of either the lack of specific diagnostic methods or the difficulty in accessing complex diagnostic methods, such as CT angiography, which have long processing times. In general, patients are admitted to emergency services during the phase in which both VAP-1 and I-FABP levels are high. From this perspective, the use of VAP-1 levels for diagnosing is as logical as the use of I-FABP levels.

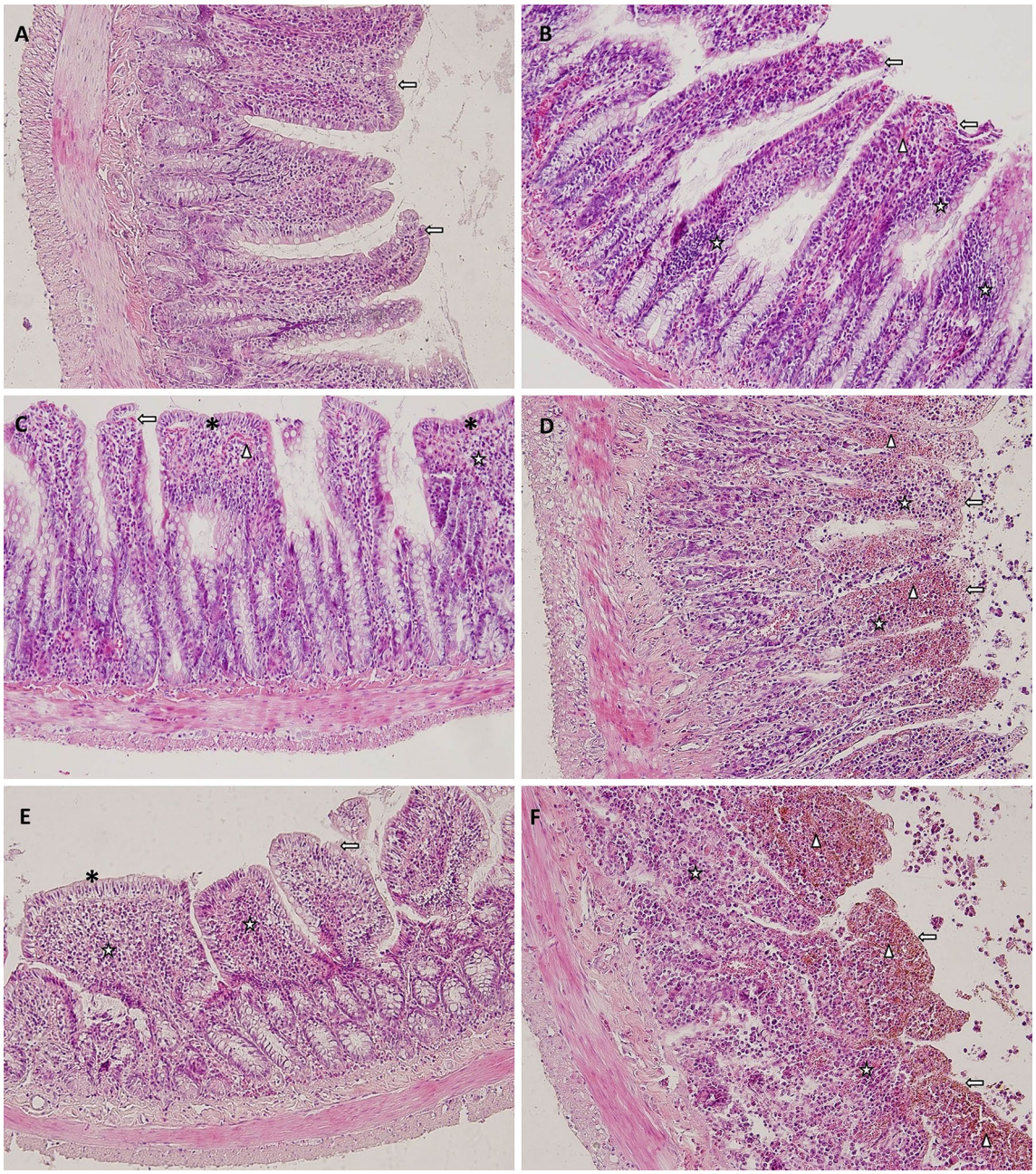

Fig. 2 Light microscopy evaluation of ileum tissues of the groups. [Photomicrograph of ileum tissue (H&E×200). Epithelium cells (ok), inflammatory cell infiltration (star), haemorrhage (arrowhead).] **a** Ileal mucosa and villus structure showing a normal morphology in Group 1. **b** Mild degeneration in the epithelial cells in Group 2, mild inflammatory cell infiltration in the lamina propria and haemorrhage. **c** Mild degeneration in the epithelium cells in Group 3; mild inflammatory cell infiltration, haemorrhage and villus fusion in the lamina propria. **d** Extensive degeneration and loss of epithelial cells in Group 4; extensive haemorrhage in the lamina propria and moderate inflammatory cell infiltration. **e** Significant villus fusion in Group 5; mild and moderate inflammatory cell infiltration in the lamina propria. **f** Significant villus damage in Group 6; acute haemorrhage and inflammatory cell infiltration in the lamina propria

In the present study, intestinal damage was also demonstrated in the control groups, especially starting from the 2-h time point. Although the damage persisted at the 6-h time point in the control groups, it did not progress. The reason for the damage is suggested to be related to inflammation in the intestinal tissue following simple laparotomy and increased intraabdominal pressure (iatrogenic intestinal damage). In the ischaemia groups, the extent of the damage increased significantly over time. Even though the damage in the ischaemia groups was more significant, the presence of intestinal damage in the control groups may have affected the significance of I-FABP and VAP-1 levels and

Table 2 The analysis of histopathologic damage scores in groups

	Time					
	30 min		2 h		6 h	
	Group 1 Control	Group 2 Ischemia	Group 3 Control	Group 4 Ischemia	Group 5 Control	Group 6 Ischemia
VAP-1[¶]						
Median	0.20[c]	0.31[e,f]	0.27[a,d]	0.90[a,e,g]	2.02[b,c,d]	4.43[b,f,g]
IQR	0.15–0.32	0.23–0.42	0.21–0.35	0.73–0.85	1.05–2.06	3.40–7.57
I-FABP[¥]						
Median	0.38[a,c,d]	1.26[a,f]	0.95[c,e]	1.37 [g]	3.13[b,d,e]	7.67[b,f,g]
IQR	0.33–0.44	0.90–1.46	1.0–1.5	0.92–2.37	2.27–4.08	6.63–11.17

[¶]a, b: Comparisons between Control and Ischemia groups according to Mann–Whitney *U* Test, $p \leq 0.05$ is statistically significant. a (Groups 3&4), $p = 0.011$; b (Groups 5&6), $p = 0.007$. c, d: Comparisons in Control groups (Groups 1,3,5) according to Mann–Whitney *U* Test with Bonferroni correction, $p < 0.016$ is statistically significant. c (Groups 1&5), $p = 0.002$; d (Groups 3&5) $p = 0.002$. e, f, g: Comparisons in Ischemia groups (Groups 2,4,6) according to Mann–Whitney *U* Test with Bonferroni correction, $p < 0.016$ is statistically significant. e (Groups 2&4), $p = 0.018$, f (Groups 2&6), $p = 0.002$; g (Groups 4&6), $p = 0.006$

[¥]a,b: Comparisons between Control and Ischemia groups according to Mann–Whitney *U* Test, $p \leq 0.05$ is statistically significant. a (Groups 1&2), $p = 0.017$; b (Groups 5&6), $p = 0.002$. c, d, e :Comparisons in Control groups (Groups 1,3,5) according to Mann–Whitney *U* Test with Bonferroni correction, $p < 0.016$ is statistically significant. c (Groups 1&3), $p = 0.013$; d (Groups 1&5), $p = 0.002$; e (Groups 3 &5), $p = 0.002$. f, g: Comparisons in Ischemia groups (Groups 2,4,6) according to Mann–Whitney *U* Test with Bonferroni correction, $p < 0.016$ is statistically significant. f (Groups 2 & 6), $p = 0.002$; g (Groups 4&6), $p = 0.003$

the significance levels of our findings. Especially in the control groups, at the 2-h time point, the involuntary intestinal damage because of handling during surgery was significant and the ischaemic intestinal damage associated with AMI had not yet peaked. Thus, I-FABP levels were likely affected by the intestinal handling and increased as a result of nonspecific damage. This could have been reason for the lack of difference between control and ischaemia groups with respect to I-FABP levels. However, the increase of I-FABP levels are approximately X2 higher in the ischemia group at 2nd hour. This might have also affected VAP-1 levels in a similar fashion; however, plasma VAP-1 levels at 2 h were significantly different between the ischaemia and control groups. In this respect, I-FABP may be considered to reflect intestinal damage related to any aetiology, and VAP-1 may be relatively more specific for ischaemia than I-FABP. At 6 h, the involuntary intestinal damage remained stable in the control group, whereas it peaked in the ischaemia group, and both I-FABP and VAP-1 levels were able to differentiate ischaemia group from the control group.

There are studies in the literature that show the pathophysiological process of VAP-1 in different pathologies such as cardiovascular diseases, ischemic—haemorrhagic stroke, arterial stiffness, inflammatory bowel diseases, obesity, diabetes and diabetes-related complications [9, 22–25]. This study is the first to demonstrate the increase in VAP-1 levels with the intestinal damage related to AMI. However, the pathophysiological process underlying the increase in VAP-1 levels in AMI should be clarified further. Acute arterial thromboembolism is one of the principal mechanisms involved in the pathophysiology of AMI ischemia. Acute thrombosis in the mesenteric circulation is frequently caused by a plaque rupture accompanying chronic atherosclerosis in the mesenteric arteries, while the mechanism involved in acute embolism is the obstruction of the mesenteric arteries by an embolism of cardiac origin. Studies have shown that VAP-1 is released into the circulation to trigger inflammation in atherosclerotic events, and particularly following vascular endothelial injury [21, 22]. Although the model in this study did not mimic the atherosclerotic cause of mesenteric ischemia, the significant increase in VAP-1 in serum, particular after the 2nd hour, may be interpreted as being intended to trigger the inflammatory process as a response to acute embolism and endothelial injury that we induced in the superior mesenteric artery. In summary, our study results show how that plasma VAP-1 and I-FABP levels increased in correlation with the time-dependent ischemia damage. I-FABP indicated ischemic damage starting at 30 min after ischaemia, whereas VAP-1 indicates ischemic damage starting at 2 h after ischemia. I-FABP levels may be superior to VAP-1 levels at early diagnosis as in the 30-min time point; however, this may not be advantageous in clinical practice because patients usually remain asymptomatic at this very early stage. Nevertheless, VAP-1 may be more useful for diagnosing AMI because VAP-1 show the highest correlation with intestinal damage and it is affected less by intestinal damage related to non-ischaemic factors. In the light of our study findings, we think that VAP-1 may be a marker capable of use in the diagnosis of mesenteric ischemia, with nonspecific findings which require advanced tests for

diagnosis. We think that if these results are now confirmed by clinical research, VAP-1, measured using ELISA kits from serum only, a minimally invasive method, can help reduce spiral CT angiography requirements by assisting with the diagnosis of mesenteric ischemia in early stages.

Limitations

The first limitation of the study is that our rat model only mimicked acute mesenteric arterial embolism and acute mesenteric arterial thrombosis. The results may be different for other types of AMI. The second limitation is the number of rats in each group. The reason why is that The Animal Care and Ethics Committee permitted seven rats only in each group depending on the welfare of animals principles. However despite this limitation, the power of our study was assessed at 80%. Third, we only evaluated the findings at 30 min, 2 h and 6 h after ischaemia. Hence, the earliest time point at which we observed that VAP-1 levels increased significantly compared to the control group was 2 h. However, VAP-1 levels might have increased at an earlier time point between 30 min and 2 h. Fourth, despite the simple laparotomy procedure, there was an involuntary but significant damage in the control group as from 2 h. During the experiment, the authors endeavoured to avoid potential confounders such as increased intraabdominal pressure and inflammation. Despite the precautions such as preventing the tight suture closure of the abdomen, involuntary intestinal damage might have affected the results of the study and the level of I-FABP also increased in the control groups. But the increase in I-FABP levels was time dependent and probably due to involuntary nonspecific damage. Additionally it was limited compared to the ischemia groups. The last limitation of the study is that VAP-1 levels were only compared to I-FABP levels which are more specific to intestinal tissue, whereas there are other markers in the literature for diagnosing AMI, such as lactate and ischemia-modified albumin.

Conclusions

Both VAP-1 and I-FABP are useful biomarkers for diagnosing, and they increase in correlation with ischaemic damage. Nevertheless, further studies are required to establish their utility in clinical practice.

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Compliance with ethical standards

Conflict of interest Aynur Sahin, Damla Aydin Altay, Selim Demir, Esin Yulug, Ahmet Menteşe, Ozgur Tatli, Yunus Karaca, Senol Ardic, Abdulkadir Gunduz and Suleyman Turedi decline that they have no conflict of interest.

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent For this type of study informed consent is not required.

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