EXPERIMENTAL STUDY

The role of heparin-binding protein in the diagnosis of acute mesenteric ischemia

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ABSTRACT

BACKGROUND: Acute mesenteric ischemia (AMI) is associated with a high mortality rate, yet diagnostic difficulties persist. Although many biomarkers have been investigated for diagnostic purposes, as well as imaging methods, a sufficiently specific and sensitive marker has not been identified. This research was designed to examine whether heparin-binding protein (HBP), which has a role in the early phase of inflammation, could be useful in the diagnosis of AMI.

METHODS: Serum samples obtained from a previously performed rabbit model of AMI were used in the study. HBP, C-reactive protein (CRP) and interleukin 6 (IL-6) levels were measured in blood samples obtained at baseline and 1, 3, and 6 hours from subjects that were separated into 3 groups: control, sham, and ischemia. The change in each marker over time and comparisons of the groups were evaluated statistically.

RESULTS: A significant difference was not detected at the first hour in any of the studied markers. At the third hour, the CRP and IL-6 levels in the ischemia group indicated a significant increase in comparison with the control and sham groups ($p<0.001$). The HBP values showed a significant increase at the sixth hour in the ischemia group in comparison with the others ($p<0.001$).

CONCLUSION: The HBP level demonstrated a slower increase in a rabbit model of AMI compared with CRP and IL-6. However, it still has the potential to become an early diagnostic biomarker. Diagnostic sensitivity and specificity should be evaluated in further clinical trials.

Keywords: Acute mesenteric ischemia; biomarker; heparin-binding protein.

INTRODUCTION

Mesenteric ischemia (MI) is less commonly observed in emergency units compared with other causes of abdominal pathology, but should be diagnosed quickly due to its high mortality risk. The most important step in the management of the disease is the diagnosis. Despite advanced radiological and surgical techniques, acute mesenteric ischemia (AMI) is still a disease with high mortality rate.[1–4]

Prognosis is significantly improved by the reinstatement of the blood flow within the first 6 hours of ischemia, and particularly embolism-related ischemia. The prognosis is worsens as the duration of intestinal ischemia is prolonged. New diagnostic methods have been investigated in order to provide early diagnosis and to thereby reduce mortality. The use of serum markers in the diagnosis of AMI is quite limited. Although many plasma markers have been investigated, no generally accepted specific marker has yet been defined.[5] In a review of 20 studies that included 18 different biochemical markers, the current markers were defined as not satisfactory. However, some new markers could contribute to diagnostic improvement.[6]

Heparin-binding protein (HBP), also known as azurocidin or CAP37, is a protein that is stored in the azurophilic granules

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of the neutrophils. Its potential role in infectious diseases has long been known and many studies have been conducted on the subject. In a recent study, HBP was demonstrated to be the only component of neutrophils that provokes increased permeability in in-vitro endothelial cell monolayers and in-vivo vascular endothelium.[7] HBP also has proinflammatory chemotactic effects against monocytes and T cells. HBP is easily mobilized within neutrophils, and this process or other functions of HBP appear to be important in early inflammation processes.[8] Clinical studies suggest HBP could be predictive in the progress of organ dysfunction, sepsis, and septic shock in patients admitted to emergency units with suspected infections and in intensive care patients.[9,10] Bacterial translocation develops in AMI, which in turn, initiates the inflammatory process. Inflammatory markers, including biomarkers, have been widely studied in AMI due to the inflammation accompanied by ischemia. The aim of this study was to analyze the blood level of HBP in AMI, which has not been studied before, and to evaluate its diagnostic potential compared with current markers. HBP may be a beneficial molecule in the early diagnosis of AMI since it has a role in the early stage of inflammation. In the present study, an experimental rabbit model of AMI was constructed and HBP, C-reactive protein (CRP) and interleukin 6 (IL-6) levels were measured at specific intervals. The change in the level of these biomarkers over time and their relationship to each other were compared statistically, and their roles in the diagnosis of AMI were evaluated.

MATERIALS AND METHODS

No new animal experiment was performed in the study. The serum samples obtained by Acar et al.[11] were used for the present research. That study was approved by the Laboratory Animals Ethical Committee of Necmettin Erbakan University Experimental Medicine Research and Practice Center on January 20, 2012 (reg. no 2012-01) and conducted at Necmettin Erbakan University Experimental Medicine Research and Practice Center in May 2012. The present study was approved by the same committee on April 17, 2013 (reg. no 2013-073). The methods applied in the original study are described in detail in the published report. As a summary:

Experimental Protocol

The study included 27 adult male and female New Zealand rabbits weighing 2500 to 3000 g. All of the animals had the same environment and nutritional conditions. Prior to the experimental study, food was withheld for 12 hours and only water was provided. The animals were classified into 3 groups: a control group of 7 rabbits, a sham group of 10 rabbits, and an ischemia group of 10 rabbits.

Control group (Group I): A dose of 50 mg/kg ketamine and 15 mg/kg xylazine was administered via the intramuscular route through the hind leg, and following attainment of anesthesia, vascular access at the dorsal ear vein of the animals was used to obtain blood samples and perform an infusion with a 22-G intravascular catheter. Blood samples of 5 mL were collected in Vacutainer gel tubes (Becton Dickinson and Co., Franklin Lakes, NJ, USA) at baseline and the first, third, and sixth hours for biochemical analysis. Following each sampling, 5 mL of 0.9% physiological saline was infused through the same catheter.

Sham group (Group II): The same procedure was applied to the rabbits in this group as in the control group. Following blood sampling for the baseline value, the abdominal region of the animals was shaved and cleaned with 10% povidone iodine. A laparotomy was performed via a midline incision, and when the peritoneum was accessed, the abdominal wall and peritoneum were closed using 2/0 silk sutures. Blood samples were collected at the first, third, and sixth hours for biochemical evaluation. Following each sampling, 5 mL of 0.9% physiological saline was infused through the same catheter, as in the control group.

Ischemia group (Group III): The same preparation that was described for the rabbits in the sham group was used in the ischemia group. Following blood sampling for the baseline value, a laparotomy was performed via midline incision, and the superior mesenteric artery was found and ligated. The peritoneum and abdominal wall were closed using 2/0 silk. Blood samples were collected at the postoperative first, third, and sixth hours for biochemical analysis. Following each sampling, 5 mL of 0.9% physiological saline was infused through the same catheter. At the end of the 6-hour ischemia period, the rabbits were sacrificed with a high dose of ketamine.

Storage of the Samples

The 5 mL blood samples collected in Vacutainer gel tubes were held for 30 minutes for coagulation, and centrifuged at 3000 rpm for 10 minutes. The serum samples were pipetted in Eppendorf tubes (Eppendorf AG, Hamburg, Germany) and kept at -80°C until biochemical analysis.

Evaluation of the Samples

Biochemical Analysis

Enzyme-linked immunosorbent assay (ELISA) tests and ready-to-use commercial kits were used for all 3 markers. The Sunred Rabbit Heparin Binding Protein (HBP) ELISA Kit (Lot No: 201090291; Shanghai Sunred Biological Technology Co., Ltd, Shanghai, China) was used for the measurement of serum HBP levels, the Scientific Research Special Eastbiopharm Rabbit CRP ELISA kit (Lot No: 20121024; Hangzhou Eastbiopharm Co. Ltd., Hangzhou, China) was used for the measurement of CRP levels, and the Cusabio Rabbit Interleukin-6 (IL-6) ELISA kit (Lot No: I4061791; Cusabio Biotech Co., Ltd, Wuhan, China) was used for measurement of IL-6 levels.

Statistical Analysis

Data were recorded on prepared forms. SPSS for Windows, Version 16.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Variance analysis with a post hoc Tukey test was used to examine the measurements between groups. A Bonferroni-corrected paired T-test was used to determine the difference between measurements. Non-normally distributed ordinary variables were analyzed using the Friedman test. A Bonferroni-corrected Mann-Whitney U test was used to determine the difference between measurements. A p value of <0.05 was accepted as statistically significant. The results were presented in tables and graphs.

RESULTS

One of the rabbits in the ischemia group died at the first hour and therefore it was excluded. The study was completed with 26 rabbits.

Biochemical Markers

CRP

The mean CRP level measured from the blood samples collected at baseline and the first, third and sixth hours in each group and the comparisons are illustrated in Table 1. The initial values were somewhat lower in the sham group than in the control group (p<0.007). No significant difference was detected between groups in the levels at the first hour (p>0.05). The CRP levels measured at the third and sixth hours revealed no significant difference between the sham and control groups (p=0.994 and p=0.932, respectively), whereas a significant increase was observed in the ischemia group compared with both the control and sham groups (p=0.0001 and p=0.0001, respectively). The change in CRP level over time is presented in Fig. 1. A progressive increase in the CRP level is notable in the sham group and particularly in the ischemia group.

IL–6

The mean IL-6 level measured from the blood samples collected at baseline and the first, third, and sixth hours in each group and the comparisons are demonstrated in Table 2. No significant difference was detected between the groups with regard to the levels at baseline and the first hour (p>0.05). The IL-6 levels measured at the third and sixth hours revealed no significant difference between the sham and control groups (p=0.882 and p=0.775, respectively), whereas a significant increase was observed in the ischemia group compared with both the control (p=0.005 and p=0.0001, respectively) and sham groups (p=0.0008 and p=0.0001, respectively) at both the third and sixth hours. The change in IL-6 level over time is presented in Fig. 2. The increase in IL-6 level in the ischemia group is notable.

HBP

The mean HBP level measured from the blood samples collected at baseline and the first, third, and sixth hours in each

Table 1. Comparison of CRP values in the groups at baseline, 1, 3, and 6 hours

	CRP	n	Mean	SD	p			95% CI
					Control	Sham	Ischemia	LL–UL
C0	Control	7	0.42	0.04	–	0.007	0.112	0.386–0.462
	Sham	10	0.35	0.05	0.007	–	0.390	0.315–0.383
	Ischemia	9	0.37	0.05	0.112	0.390	–	0.342–0.411
	Total	26	0.38	0.05	–	–	–	0.357–0.400
C1	Control	7	0.49	0.10	–	0.075	0.879	0.368–0.609
	Sham	10	0.39	0.02	0.075	–	0.150	0.361–0.422
	Ischemia	9	0.47	0.08	0.879	0.150	–	0.408–0.527
	Total	26	0.44	0.09	–	–	–	0.407–0.481
C3	Control	7	0.42	0.06	–	0.994	<0.001	0.367–0.469
	Sham	10	0.42	0.03	0.994	–	<0.001	0.398–0.444
	Ischemia	9	0.52	0.05	<0.001	<0.001	–	0.484–0.567
	Total	26	0.45	0.07	–	–	–	0.429–0.484
C6	Control	7	0.44	0.07	–	0.932	<0.001	0.374–0.506
	Sham	10	0.45	0.04	0.932	–	<0.001	0.423–0.475
	Ischemia	9	0.62	0.05	<0.001	<0.001	–	0.586–0.658
	Total	26	0.50	0.10	–	–	–	0.466–0.546

CI: Confidence interval; CRP: C-reactive protein; LL: Lower limit; SD: Standard deviation; UL: Upper limit.

Table 2. Comparison of IL-6 values in the groups at baseline, 1, 3, and 6 hours

	IL-6	n	Mean	SD	p			95% CI
					Control	Sham	Ischemia	LL–UL
I0	Control	7	9.26	3.10	–	0.729	0.988	6.390–12.124
	Sham	10	7.78	2.73	0.729	–	0.597	5.829–9.733
	Ischemia	9	9.55	5.37	0.988	0.597	–	5.423–13.674
	Total	26	8.79	3.86	–	–	–	7.232–10.348
I1	Control	7	10.17	3.78	–	0.999	0.360	6.679–13.664
	Sham	10	10.10	3.52	0.999	–	0.280	7.584–12.617
	Ischemia	9	13.22	5.44	0.360	0.280	–	9.044–17.407
	Total	26	11.20	4.43	–	–	–	9.413–12.989
I3	Control	7	7.91	3.93	–	0.882	0.005	4.276–11.553
	Sham	10	10.10	4.18	0.882	–	0.008	7.110–13.093
	Ischemia	9	24.28	14.73	0.005	0.008	–	12.963–35.601
	Total	26	14.42	11.56	–	–	–	9.751–19.092
I6	Control	7	7.68	4.66	–	0.775	<0.001	3.376–11.996
	Sham	10	10.65	3.37	0.775	–	<0.001	8.242–13.057
	Ischemia	9	33.10	13.91	<0.001	<0.001	–	22.413–43.795
	Total	26	17.62	14.30	–	–	–	11.847–23.402

CI: Confidence interval; IL-6: Interleukin 6; LL: Lower limit; SD: Standard deviation; UL: Upper limit.

Table 3. Comparison of HBP values in the groups at baseline, 1, 3, and 6 hours

	HBP	n	Mean	SD	p			95% CI
					Control	Sham	Ischemia	LL–UL
H0	Control	7	6.22	2.58	–	0.696	0.397	3.834–8.612
	Sham	10	5.35	1.46	0.696	–	0.072	4.309–6.394
	Ischemia	9	7.66	2.45	0.397	0.072	–	5.779–9.548
	Total	26	6.38	2.31				5.455–7.317
H1	Control	7	5.13	1.90	–	0.414	0.617	3.383–6.888
	Sham	10	3.90	2.38	0.414	–	0.059	2.194–5.601
	Ischemia	9	6.46	2.56	0.617	0.058	–	4.496–8.431
	Total	26	4.91	2.99				3.704–6.123
H3	Control	7	6.30	2.79	–	0.959	0.345	3.718–8.881
	Sham	10	6.64	2.14	0.959	–	0.427	5.111–8.170
	Ischemia	9	8.10	2.68	0.345	0.427	–	6.047–10.159
	Total	26	7.05	2.54				6.031–8.079
H6	Control	7	5.13	1.33	–	0.592	0.012	3.900–6.360
	Sham	10	4.08	1.34	0.592	–	<0.001	3.122–5.043
	Ischemia	9	8.54	3.15	0.012	<0.001	–	6.115–10.964
	Total	26	5.91	2.87				4.747–7.068

CI: Confidence interval; HBP: Heparin-binding protein; LL: Lower limit; SD: Standard deviation; UL: Upper limit.

group and the comparisons are provided in Table 3. No significant difference was detected between groups in the levels at baseline or the first and third hours (p>0.05). A significant increase was observed in the HBP level of the ischemia group compared with both the control and sham groups at the sixth hour (p=0.0012 and p=0.0001, respectively). The change in

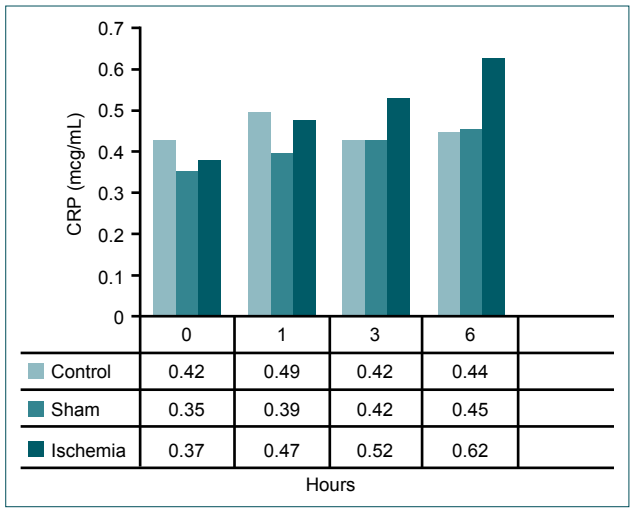

Figure 1. Time course of serum C-reactive protein levels in the control, sham, and ischemia groups.

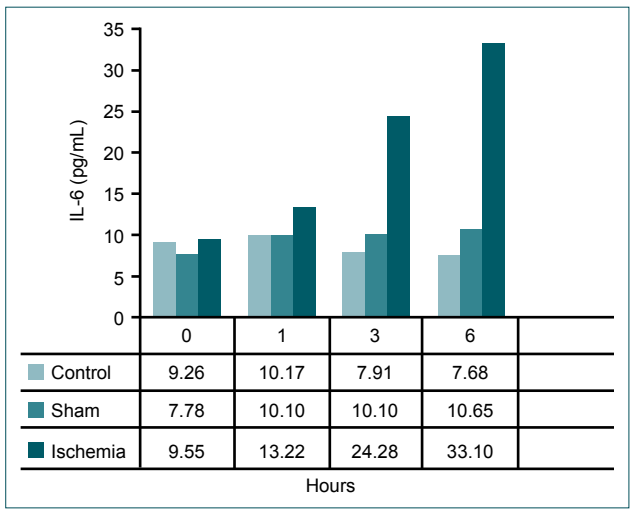

Figure 2. Time course of serum interleukin 6 levels in the control, sham, and ischemia groups.

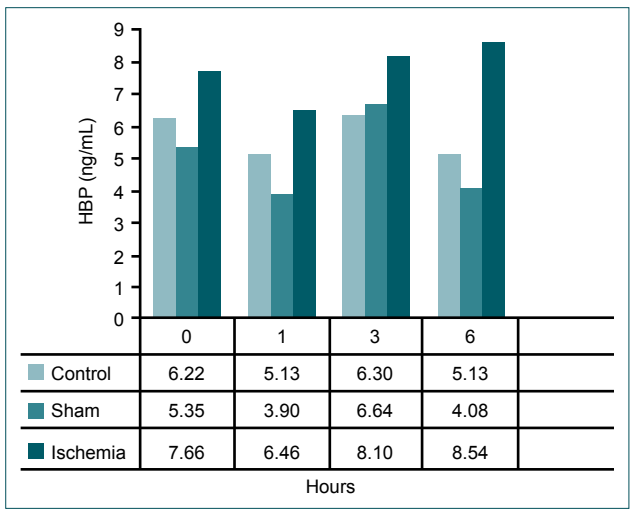

Figure 3. Time course of serum heparin-binding protein levels in the control, sham, and ischemia groups.

HBP level over time is presented in Fig. 3. The increase in HBP level over time was less significant compared with the increase in CRP and IL-6 levels.

DISCUSSION

The clinical findings of AMI arise from impaired perfusion, systemic inflammatory responses due to impaired microcirculation, and reperfusion damage.[12] At the cellular stage, ischemia leads to mitochondrial dysfunction, impaired ion transport, and intracellular acidosis. Alterations in membrane permeability and the secretion of free radicals and disintegrating enzymes result in cell death and necrosis.[12,13] Many cells within the ischemic tissue, such as neutrophils, endothelial cells, monocytes, and platelets are activated. Many proinflammatory substances, including tumor necrosis factor (TNF), interleukins, platelet activating factor, and leukotrienes are secreted. As a result, the damage is related to leukocyte adhesion, platelet aggregation, and impaired nitric oxide production.[14] It has long been known that ischemia leads to bacterial translocation due to changes in vascular permeability within a short time.[15,16] In this study, we investigated the possibility that HBP, which has been demonstrated to have antibacterial effects as well as effects on both inflammation and vascular permeability, could be a beneficial marker for early diagnosis of AMI. The animals in the ischemia group were found to have significantly higher HBP levels at the sixth hour of ischemia compared with those in the sham and control groups.

HBP is a member of the serin protease family.[17] Although it has no enzymatic activity, it is a multi-functional protein. It accumulates and activates monocytes, induces T cells, starts detachment of endothelial cells and fibroblasts and the homotypic accumulation of these cells. Furthermore, endocytosis of HBP into monocytes increases the production of lipopolysaccharide-derived TNF-α.[8] Other studies have suggested that internalized HBP protects endothelial cells against apoptosis.[18] HBP has been shown to play a specific role in mediating the change in vascular permeability that is stimulated by chemoattractant-induced polymorphonuclear leukocyte activation.[7] The localization of HBP duplicates within azurophilic granules and secreted vesicles indicates a possible role of the protein in neutrophils. The HBP localized in azurophilic granules is in close contact with internalized bacteria after the azurophil granules are fused with the phagosome. Again, the HBP secreted from the secretory vesicles has been reported to have important functions during early stages of inflammation.

Unfortunately, in more than 20 studies conducted of biomarkers, no ideal biomarker with a high sensitivity and specificity has been detected for the diagnosis of AMI. The serum phosphate level is one of the biochemical parameters researched for diagnostic value in AMI. In a study conducted on 20 rabbits, the serum phosphate levels were shown to increase

in AMI.[19] Feretis et al.[20] demonstrated significantly higher serum phosphate levels in 18 patients with acute intestinal infarction compared with 24 patients without intestinal ischemia. In an intestinal ischemia study conducted on dogs, a significant increase in the phosphate level was observed in the fourth hour of ischemia. However, the authors noted that this had no benefit in early diagnosis, and that the increase in phosphate level was meaningful following the development of irreversible necrosis within the intestines.[21] Leo et al.[22] found in a study of 23 AMI patients that serum phosphate levels had no diagnostic or prognostic value.

Another marker used in AMI is amylase. It has been reported that amylase levels were found to be higher than normal in 27 of 52 patients diagnosed with AMI.[23] In a study that included patients with acute abdomen on admission and who were diagnosed with AMI that amylase was 25% specific and 63% sensitive.[24] In another study, the amylase levels of the patients with AMI were observed to be high starting at the third hour.[25] Amylase has been demonstrated to increase in many disorders included in the etiology of acute abdomen. Its single usage in AMI is controversial, however, and should be confirmed with other parameters. Alone, the sensitivity and specificity are low.

In the experimental study conducted by Kurt et al.,[26] the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of D-dimer in AMI were found to be 88.8%, 90%, 88.8%, and 100%, respectively, and they concluded that D-dimer may be beneficial in the early diagnosis of AMI. In another experimental AMI study, procalcitonin (PCT) was demonstrated to significantly increase in blood starting in the first hour of ischemia.[27] In a study investigating the PCT level in intestinal strangulation, PCT levels were higher than normal in the group with strangulation. PCT levels were observed to increase at the 30th and 60th minutes of the study, and a significant increase was detected in the 120th minute.[28]

In a series that included 7 patients with AMI, the ischemia-modified albumin (IMA) level were observed to be significantly higher than that of healthy controls.[29] In another clinical study, the IMA level was measured in 26 patients, 12 of whom were diagnosed to have intestinal ischemia, and the IMA level was significantly elevated. High IMA levels were reported to have 100% sensitivity and 100% specificity in the diagnosis of intestinal ischemia.[30] In a study conducted on rabbits, the IMA level was found to significantly increase with additional ischemia time.[31]

In a study of 61 patients with acute abdominal pain, serum intestinal fatty acid binding protein (IFABP) levels were significantly higher in cases with ischemic bowel disease.[32] In another study, true positivity was detected for high IFABP in 7 patients with intestinal ischemia, whereas false positivity was detected in 10.[33] Another study examined 21 patients with strangulated intestinal obstruction, 3 of whom had intestinal necrosis. The serum IFABP level was high in all of the patients with intestinal necrosis.[34] A meta-analysis determined that the sensitivity and specificity of the serum IFABP level in the diagnosis of intestinal ischemia was 72% (51–88%) and 73% (62–83%), respectively.[6] In the study conducted by Dundar et al.,[35] no significant difference was detected between the serum IFABP level in control, sham, and ischemia groups. Furthermore, no significant difference was observed at the initial, first, third, and sixth hours of ischemia in the ischemia group.

A literature search revealed no study on the diagnostic or prognostic value of HBP on patients with AMI. The number of clinical studies on HBP is limited. Considering its effect on vascular permeability, studies investigating whether it could be a marker of acute respiratory or circulatory failure in serious infections and sepsis have become important.

Chew et al.[36] investigated the HBP level in 53 patients with septic and non-septic shock in the emergency unit, The HBP level was significantly higher among patients with shock compared with that of healthy or control patients with an infection (such as urinary infection, pneumonia, gastroenteritis), whereas no difference was observed between patients with septic and non-septic shock. Research performed by Linder et al.[9] investigating the predictive value of HBP in the progression to circulatory failure or septic shock in patients with a suspected infection or sepsis, the HBP level in patients with serious sepsis or septic shock was significantly higher compared with that of patients with non-serious sepsis. PCT, white blood cell count (WBC), CRP, IL-6, and lactate values were used for comparison in predicting the progression to serious sepsis and septic shock and were found to be lower than that of HBP. The sensitivity, specificity, NPD, PPD and receiver operating characteristic (ROC) curve values of HBP were higher than those of other markers. In another study, Linder et al.,[10] investigated 759 patients admitted to emergency units with a suspected infection. In all, 674 were diagnosed with various infections, and among that group, 487 had no organ dysfunction at admission. During the study period (72 hours), 141 of the 487 developed organ dysfunction. The authors reported that blood samples were collected for measurement of HBP, WBC, PCT, CRP and lactate on admission and within 12 to 24 hours after admission and compared for their predictive value for serious sepsis and organ dysfunction. HBP was superior to the other markers in patients with organ failure both on admission and during the study in terms of sensitivity, specificity, NPD, PPD, and ROC values. HBP was reported to increase hours before organ dysfunction (median: 10.5 hours) and to have a higher odds ratio (OR: 20.5) for indicating progression to organ dysfunction in comparison with the other markers.

In another study investigating the relationship between reduced oxygenation and circulatory failure and the HBP level in intensive care patients, the HBP level on admission was

found to be related to respiratory and circulatory failure and 30-day mortality.[37]

In an experimental study using CRP as a marker in intestinal ischemia, CRP was found to be more elevated in rats with bacterial translocation.[38] CRP concentrations were also observed to increase parallel with the severity of tissue damage or inflammation in acute intensive infection.[39] In an AMI model study conducted on pigs, the increase in total microflora and bacterial translocation was determined to be consistent with the increase in CRP.[40] Willetts et al.[41] reported high CRP levels in children with acute endotoxinemia with acute invagination, and the finding was correlated to the severity of the disease. Studies of IL-6 have revealed that systemic secretion of TNF-α and IL-6 was related to septic shock and mortal outcomes. It has been reported in a study that TNF-α and IL–6 levels continuously increased following intestinal ischemia, and that these cytokines were secreted from Kuppfer cells.[42] The increase in blood levels of IL-6 in AMI is valuable in demonstrating the early stage onset of systemic response and evaluating the clinical situation of the patients, rather than diagnosing the disease. Another experimental study that compared hemorrhagic shock and intestinal ischemia, no correlation was observed between bacterial translocation and blood levels of cytokines, and IL–6 levels were demonstrated to peak in the third hour after superior mesenteric artery occlusion.[43] In our study, the significant increases in both the CRP and IL-6 levels in the ischemia group were also observed in the third hour, which was earlier than the increase in HBP. However, it should be considered that both CRP and IL-6 are highly non-specific markers and are elevated in many ischemic and inflammatory conditions. Therefore, it is important to note the diagnostic sensitivity and specificity in addition to the ability to be detected in blood at early stages, because the markers studied so far have not been found to be sufficiently sensitive and specific. Since our study was an animal study and included a limited sample, the specificity and sensitivity tests would not produce acceptable statistical results.

Conclusion

This study investigated whether or not HBP, which has been known to have antibacterial effects as well as effects on the early stages of inflammation and vascular permeability, was a beneficial marker in the early diagnosis of acute mesenteric ischemia. Furthermore, the HBP level was compared to the levels of IL-6, which is an important cytokine in inflammatory conditions, and CRP, which is an acute phase reactant induced by IL-6. Although detected later than IL-6 and CRP, a significant increase in the HBP level in the ischemia group in the sixth hour has demonstrated that it could be a potential biomarker in the diagnosis of AMI. However, in order to define the specificity and sensitivity of HBP for AMI, and to determine a cutoff value, clinical studies with larger sample sizes are needed. Combination studies conducted with other markers should be considered as factors to increase the specificity and sensitivity.

Conflict of interest: None declared.

REFERENCES

1. Yasuhara H. Acute mesenteric ischemia: the challenge of gastroenterology. Surg Today 2005;35:185–95.
2. Ujiki M, Kibbe MR. Mesenteric ischemia. Perspect Vasc Surg Endovasc Ther 2005;17:309–18.
3. Oldenburg WA, Lau LL, Rodenberg TJ, Edmonds HJ, Burger CD. Acute mesenteric ischemia: a clinical review. Arch Intern Med 2004;164:1054–62.
4. Chang JB, Stein TA. Mesenteric ischemia: acute and chronic. Ann Vasc Surg 2003;17:323–8.
5. Acosta S, Nilsson TK, Björck M. D-dimer testing in patients with suspected acute thromboembolicocclusion of the superior mesenteric artery. Br J Surg 2004;91:991–4.
6. Evennett NJ, Petrov MS, Mittal A, Windsor JA. Systematic review and pooled estimates for the diagnostic accuracy of serological markers for intestinal ischemia. World J Surg 2009;33:1374–83.
7. Gautam N, Olofsson AM, Herwald H, Iversen LF, Lundgren-Akerlund E, Hedqvist P, et al. Heparin-binding protein (HBP/CAP37): a missing link in neutrophil-evoked alteration of vascular permeability. Nat Med 2001;7:1123–7.
8. Tapper H, Karlsson A, Mörgelin M, Flodgaard H, Herwald H. Secretion of heparin-binding protein from human neutrophils is determined by its localization in azurophilic granules and secretory vesicles. Blood 2002;99:1785–93.
9. Linder A, Christensson B, Herwald H, Björck L, Akesson P. Heparin-binding protein: an early marker of circulatory failure in sepsis. Clin Infect Dis 2009;49:1044–50.
10. Linder A, Arnold R, Boyd JH, Zindovic M, Zindovic I, Lange A, et al. Heparin-Binding Protein Measurement Improves the Prediction of Severe Infection With Organ Dysfunction in the Emergency Department. Crit Care Med 2015;43:2378–86.
11. Acar T, Koçak S, Cander B, Ergin M, Dikmetaş C. Liporotein-associated phospholipase-A2 can be a diagnostic marker inthe early stage diagnosis of acute mesenteric ischemia. Turk J Med Sci 2016;46:120–5.
12. Abboud B, Daher R, Boujaoude J. Acute mesenteric ischemia after cardio-pulmonary bypass surgery. World J Gastroenterol 2008;14:5361–70.
13. Cerqueira NF, Hussni CA, Yoshida WB. Pathophysiology of mesenteric ischemia/reperfusion: a review. Acta Cir Bras 2005;20:336–43.
14. Harward TR, Brooks DL, Flynn TC, Seeger JM. Multiple organ dysfunction after mesenteric artery revascularization. J Vasc Surg 1993;18:459–67.
15. Necefli A, Dolay K, Arikan Y, Guloglu R, Karayay S, Halici E, et al. The effect of ceftriaxone on bacterial translocation in mesenteric ischemia. Ulus Travma Acil Cerrahi Derg 1999;5:7–10.
16. Deitch EA. Bacterial translocation or lymphatic drainage of toxic products from the gut: what is important in human beings? Surgery 2002;131:241–4.
17. Pereira HA. CAP37, a neutrophil-derived multifunctional inflammatory mediator. J Leukoc Biol 1995;57:805–12.
18. Olofsson AM, Vestberg M, Herwald H, Rygaard J, David G, Arfors KE, et al. Heparin-binding protein targeted to mitochondrial compartments protects endothelial cells from apoptosis. J Clin Invest 1999;104:885–94.
19. Uncu H, Uncu G, İlçöl Y, Aker Y. Diagnosis of intestinal ischemia by measurement of serum phosphate and enzyme changes and the effectiveness of vitamin E treatment. Turk J Gastroenterol 1999;10:272–5.
20. Feretis CB, Koborozos BA, Vyssoulis GP, Manouras AJ, Apostolidis NS,

Golematis BC. Serum phosphate levels in acute bowel ischemia. An aid to early diagnosis. Am Surg 1985;51:242–4.
21. Lores ME, Cañizares O, Rosselló PJ. The significance of elevation of serum phosphate levels in experimental intestinal ischemia. Surg Gynecol Obstet 1981;152:593–6.
22. Leo PJ, Simonian HG. The role of serum phosphate level and acute ischemic bowel disease. Am J Emerg Med 1996;14:377–9.
23. Wilson C, Gupta R, Gilmour DG, Imrie CW. Acute superior mesenteric ischaemia. Br J Surg 1987;74:279–81.
24. Delaney CP, O'Neill S, Manning F, Fitzpatrick JM, Gorey TF. Plasma concentrations of glutathione S-transferase isoenzyme are raised in patients with intestinal ischaemia. Br J Surg 1999;86:1349–53.
25. Aslan A,Temiz M, Semerci E, Özkan OV. Acute Mesenteric Ischemia: Clinical Experience. Eurasian J Emerg Med 2009;8:28–32.
26. Kurt Y, Akin ML, Demirbas S, Uluutku AH, Gulderen M, Avsar K, et al. D-dimer in the early diagnosis of acute mesenteric ischemia secondary to arterial occlusion in rats. Eur Surg Res 2005;37:216–9.
27. Karabulut K, Gül M, Dündar ZD, Cander B, Kurban S, Toy H. Diagnostic and prognostic value of procalcitonin and phosphorus in acute mesenteric ischemia. Ulus Travma Acil Cerrahi Derg 2011;17:193–8.
28. Papaziogas B, Anthimidis G, Koutelidakis I, Atmatzidis S, Atmatzidis K. Predictive value of procalcitonin for the diagnosis of bowelstrangulation. World J Surg 2008;32:1566–7.
29. Gunduz A, Turedi S, Mentese A, Karahan SC, Hos G, Tatli O, et al. Ischemia-modified albumin in the diagnosis of acute mesenteric ischemia: a preliminary study. Am J Emerg Med 2008;26:202–5.
30. Polk JD, Rael LT, Craun ML, Mains CW, Davis-Merritt D, Bar-Or D. Clinical utility of the cobalt-albumin binding assay in the diagnosis of intestinal ischemia. J Trauma 2008;64:42–5.
31. Dundar ZD, Cander B, Gul M, Karabulut KU, Girisgin S. Serum ischemia-modified albumin levels in an experimental acute mesenteric ischemia model. Acad Emerg Med 2010;17:1233–8.
32. Kanda T, Fujii H, Tani T, Murakami H, Suda T, Sakai Y, et al. Intestinal fatty acid-binding protein is a useful diagnostic marker for mesenteric infarction in humans. Gastroenterology 1996;110:339–43.
33. Lieberman JM, Sacchettini J, Marks C, Marks WH. Human intestinal fatty acid binding protein: report of an assay with studies in normal volunteers and intestinal ischemia. Surgery 1997;121:335–42.
34. Cronk DR, Houseworth TP, Cuadrado DG, Herbert GS, McNutt PM, Azarow KS. Intestinal fatty acid binding protein (I-FABP) for the detection of strangulated mechanical small bowel obstruction. Curr Surg 2006;63:322–5.
35. Dundar ZD, Cander B, Gul M, Karabulut KU, Kocak S, Girisgin S, et al. Serum intestinal fatty acid binding protein and phosphate levels in the diagnosis of acute intestinal ischemia: an experimental study in rabbits. J Emerg Med 2012;42:741–7.
36. Chew MS, Linder A, Santen S, Ersson A, Herwald H, Thorlacius H. Increased plasma levels of heparin-binding protein in patients with shock: a prospective, cohort study. Inflamm Res 2012;61:375–9.
37. Tydén J, Herwald H, Sjöberg F, Johansson J. Increased Plasma Levels of Heparin-Binding Protein on Admission to Intensive Care Are Associated with Respiratory and Circulatory Failure. PLoS One 2016;11:e0152035.
38. Cevikel MH, Ozgün H, Boylu S, Demirkiran AE, Aydin N, Sari C, et al. C-reactive protein may be a marker of bacterial translocation in experimental intestinal obstruction. ANZ J Surg 2004;74:900–4.
39. Johnson HL, Chiou CC, Cho CT. Applications of acute phase reactants in infectious diseases. J Microbiol Immunol Infect 1999;32:73–82.
40. Meddah AT, Leke L, Romond MB, Grenier E, Cordonnier C, Risbourg B, et al. The effects of mesenteric ischemia on ileal colonization, intestinal integrity, and bacterial translocation in newborn piglets. Pediatr Surg Int 2001;17:515–20.
41. Willetts IE, Kite P, Barclay GR, Banks RE, Rumley A, Allgar V, et al. Endotoxin, cytokines and lipid peroxides in children with intussusception. Br J Surg 2001;88:878–83.
42. Towfigh S, Heisler T, Rigberg DA, Hines OJ, Chu J, McFadden DW, et al. Intestinal ischemia and the gut-liver axis: an in vitro model. J Surg Res 2000;88:160–4.
43. Grotz MR, Ding J, Guo W, Huang Q, Deitch EA. Comparison of plasma cytokine levels in rats subjected to superior mesenteric artery occlusion or hemorrhagic shock. Shock 1995;3:362–8.

DENEYSEL ÇALIŞMA - *ÖZET*

Akut mezenter iskemi tanısında heparin-bağlayıcı proteinin rolü

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AMAÇ: Akut mezenterik iskemi (AMİ), acil servislere sık başvuru nedenlerinden biri değildir. Ancak tanısal zorluklar ve bununla ilişkili yüksek mortalite düzeyleri sürmektedir. Görüntüleme yöntemleri yanı sıra birçok biyokimyasal belirteç tanısal amaçlı araştırılmış olmasına rağmen yeterince özgül ve duyarlı bir belirteç ortaya konamamıştır. Bu çalışmada, enflamasyonun erken safhasında rol oynadığı belirlenen heparin-bağlayıcı proteinin (HBP) AMİ tanısında yararlı olup olamayacağı araştırıldı.
GEREÇ VE YÖNTEM: Çalışmada daha önce yapılmış, AMİ'nin bir tavşan modelinden elde edilmiş serum örnekleri kullanıldı. Kontrol, sham ve iskemi grubu olmak üzere üç gruba ayrılan deneklerden 0., 1., 3. ve 6. saatlerde elde edilen kan örneklerinde HBP, C-reaktif protein (CRP) ve interlökin-6 (IL-6) düzeyleri ölçüldü. Herbir belirtecin zamanla değişimi ve biribirleriyle karşılaştırılması istatistiksel olarak değerlendirildi.
BULGULAR: Çalışılan belirteçlerin hiçbirinde 1. saatte gruplar arasında anlamlı bir fark belirlenmedi. Üçüncü saatten itibaren iskemi grubunda CRP ve IL-6 düzeyleri, kontrol ve sham gruplarına göre anlamlı yükselme göstermiştir ($p<0.001$). HBP değerleri ise 6. saatten itibaren iskemi grubunda diğerlerine göre anlamlı yükselme gösterdi ($p<0.001$).
TARTIŞMA: Heparin-bağlayıcı protein düzeyleri, AMİ'nin tavşan modelinde CRP ve IL-6'ya göre daha geç yükselme gösterdi. Ancak yine de bir erken tanı belirteci olma potansiyel taşımaktadır. Yapılacak klinik çalışmalarla tanısal sensitivite ve spesifisitesinin değerlendirilmesi gerekmektedir.
Anahtar sözcükler: Akut mezenter iskemi; biyobelirteç; heparin-bağlayıcı protein.

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