EXPERIMENTAL STUDY

The importance of serum intestinal fatty acid-binding protein for the early diagnosis of acute mesenteric ischemia

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ABSTRACT

BACKGROUND: Acute mesenteric ischemia (AMI), although relatively rare, is an emergency condition with high mortality rates (60–80%) attributed to lack of early diagnosis. The aim of this experimental study was to observe the changes in serum intestinal fatty acid-binding protein (I-FABP) levels over time in the AMI model by ligating superior mesenteric artery (SMA) in rats and to compare with the serum I-FABP levels of the rats in the control group.

METHODS: Twenty rats were randomly allocated into two groups as control and ischemia group. The basal serum I-FABP levels were determined. SMA was isolated by laparotomy in all animals. In the ischemia group, SMA was ligated and intestinal ischemia was formed. Blood was taken from each rat in both groups at 30th, 60th, and 90th min to determine the serum I-FABP levels. The blood results were compared between two groups and were also compared by time in each group.

RESULTS: In the ischemia group, serum I-FABP levels were significantly higher than the control group at post-operative 30th, 60th, and 90th min ($p<0.01$). In comparison with pre-operative serum I-FABP levels, remarkable increases were observed statistically at post-operative 30th, 60th, and 90th min in the ischemia group ($p<0.01$). In contrast, there was no statistically significant difference within the serum I-FABP levels over time in the control group. The increases of serum I-FABP levels in the ischemia group were directly correlated with the time of ischemia.

CONCLUSION: Serum I-FABP levels have increased significantly in the intestinal ischemia and these values have risen progressively over time. Serum I-FABP may be a useful and promising biomarker for the early diagnosis of AMI.

Keywords: Acute mesenteric ischemia; biomarker; early diagnosis; intestinal fatty acid-binding protein.

INTRODUCTION

Acute mesenteric ischemia (AMI) is an emergency with a relatively low incidence rate, but has a high mortality rate (60–80%) because of failure to diagnose early.[1–5] The causes of AMI are mesenteric arterial embolism, mesenteric arterial thrombosis, mesenteric venous thrombosis, or non-occlusive intestinal mesenteric ischemia.

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Early diagnosis of AMI is very important for treatment, but it remains difficult. Clinical symptoms and physical examination are not reliable to differentiate AMI from other causes of abdominal pain.[6,7] There is not currently an effective laboratory test or radiologic diagnostic tests for the early diagnosis.[8] High levels of amylase, aspartate aminotransferase, lactate dehydrogenase (LDH), and creatine phosphokinase may be evaluated, none of these tests are sensitive or specific.[9] Numerous studies have examined serum levels of biological indicators such as D-dimer, alpha-glutamate S-transferase, D-lactate, L-lactate, LDH, and serum phosphate in early diagnosis of AMI, all of these tests remain controversial in regard to the significance of these indicators in early diagnosis of intestinal ischemia.[10–14]

Fatty acid-binding protein (FABP) is a small (12–15 kDa) intracellular protein. FABP is bound to fatty acids and plays a role in carrying these fatty acids. FABP also is important for protecting of cellular fatty acids from unfavorable effects.[15] There are immunologically different types of FABPs, which are found in tissues such as the heart, intestines, liver, epidermis, muscle, adipose, and three other types. The occurrence of FABP at cell level is decided at the transcriptional level. This level increases with different pathophysiological and pharmacological effects, such as ischemia. Moreover, one type of FABP may be found in more than one organ.[15]

Intestinal fatty acid-binding protein (I-FABP) is present in the epithelium cells of the stomach, small intestine, and large intestine. I-FABP is rapidly released into the circulatory system when intestinal mucosal injury consists. I-FABP may represent a potential serum marker for the diagnosis of intestinal disease due to its low molecular mass and specific intestinal localization.[16]

Some studies have shown that elevation of serum I-FABP levels may indicate small bowel epithelial damage such as mesenteric ischemia, strangulated obstruction of the small bowel, and necrotizing enterocolitis.[17–19] However, I-FABP is still not certainly available as a biomarker for AMI in clinical practice.

We hypothesized that serum I-FABP levels may increase, when we create AMI by mesenteric artery occlusion in rats. The aim of this experimental study was to observe the changes in serum I-FABP levels over time in the AMI model by binding superior mesenteric artery (SMA) in rats and to compare with the serum I-FABP levels of the rats in the control group.

MATERIALS AND METHODS

Experimental Study Design and Setting

This was a randomized, controlled animal study. All experimental protocols were approved by the Yeditepe University Experiment Animals Ethics Committee (Ref. no: 2011-164). In our study, we used 20 male Wistar-Albino rats (weighing approximately 350–400 g) obtained from Yeditepe University Experimental Study Laboratory. All operations were carried out pursuant to anesthesia and animal care methods, universally accepted guidelines on the care and use of laboratory animals (National Institutes of Health Guidelines on the Care and Use of Laboratory Animals). All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Experimental Protocol

All rats involved in the study were divided into two randomized groups. The 20 rats were numbered from 1 to 20 and were randomly allocated into two groups in equal number. Two groups were specified as follows:

Control Group

Pre-operative blood was taken from rats in this group and basal values were found. Then laparotomy was performed. After the laparotomy, SMA was simply manipulated and blood samples were taken 30th, 60th, and 90th min.

Ischemia Group

Pre-operative blood was taken from rats in this group and basal values were found. Then, laparotomy was performed. After laparotomy, SMA was ligated and intestinal ischemia was formed. Then, blood samples were taken 30th, 60th, and 90th min.

Operation Details

After a night fasting, anesthesia was applied on the rats using 50 mg/kg intramuscular ketamine (Ketalar; Eczacıbaşı Pharmaceutical and Trada Inc. Istanbul - Turkey) and 10 mg/kg xylazine (Rompun; Bayer Turk Chemistry Industry Ltd. Co. Istanbul - Turkey). The rats were allowed to spontaneously breathe during operation. A heating lamp was used to maintain a body temperature of 37°C. 10 ml Ringer Lactate solutions were given to the rats subcutaneously, to prevent dehydration at the end of operation. The abdominal surface was shaved and cleaned twice with 10% Povidone-iodine solution, and then the operation was started utilizing sterile equipment pursuant to sterile technique.

Blood samples were taken before the operation from dorsal and lateral tail vens of all rats, then laparotomy was performed. In the control group, SMA was isolated and manipulated but not bound (Fig. 1a). In the ischemia group, SMA was ligated with 3/0 silk suture from the origin of the aorta and intestinal ischemia was formed. Abdominal incisions were covered with 3–0 polyglactin sutures. 0.5 ml of blood samples were taken from tail veins of each rats at post-operative 30th, 60th, and 90th min to measure serum I-FABP levels. The post-operative 90th min, samples were received for pathologic examination from ischemic intestinal segment by re-laparotomy in the ischemia group (Fig. 1b). At the end of the experiment, excessive doses of anesthesia were given to all rats

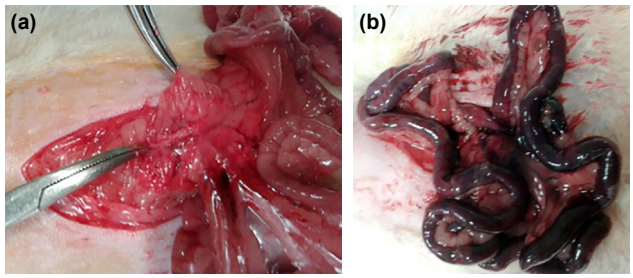

Figure 1. (a) The exploration of superior mesenteric artery. (b) The image of mesenteric ischemia at post-operative 90th min in the rats of ischemia group.

and euthanasia was performed. The serum I-FABP levels were compared between the two groups and were also compared by time in each group.

Storage of the Samples

Individual samples of 0.5 ml blood were placed into dry tubes and were kept for 30 min to allow coagulation, and then these samples were centrifuged at 1000× g for 15 min. Serum samples obtained were pipetted and placed in Eppendorf tubes. Samples were kept at (–) 20°C until biochemical examination. For histopathologic examination, 2 cm distal ileum samples were washed with serum physiologic and then fixed with 10% formaldehyde and were subsequently placed in paraffin blocks after routine xylol-alcohol series.

Evaluation of the Samples

Samples from the rats have examined at Dr. Lütfi Kırdar Kartal Training and Research Hospital Biochemistry Department Elisa Laboratory. To detect serum I-FABP level, we used HC 3101 coded Rat I-FABP Elisa test kit of Hycult Biotechnology. Samples were examined at Biochemistry Department with Biotek ELX 800 ELİSA device. The study was carried out pursuant to the protocol of the producer company.

Histopathologic examinations were performed in the pathology department. Small intestine samples obtained from rats in the ischemia group were fixed in 10% formaldehyde and were evaluated by the pathologist.

Statistical Analysis

Study findings and statistical analysis were examined utilizing Number Cruncher Statistical System 2007 and PASS 2008 Statistical Software (Utah, USA) program. While study data were being evaluated, student t test was used for comparison of normal distributed parameters between two groups, and variance analysis in recurring measurements was used for comparison within the groups. Significance was determined to be $p<0.05$.

RESULTS

The study was completed with 20 rats, ten of which underwent mesenteric ischemia, and ten of which were designated to the operated control group. There was no statistically significant difference in terms of pre-operative serum I-FABP levels between two groups ($p>0.05$); however, 30th, 60th, and 90th min serum I-FABP levels in the ischemia group were found significantly higher than the control group ($p<0.01$) (Table 1). Serum I-FABP levels significantly elevated at 30th min of ischemia.

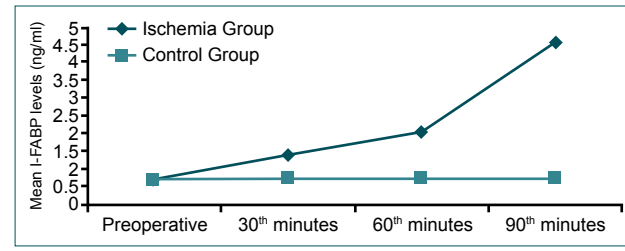

Figure 2. Comparison of changes in serum I-FABP levels over time between two groups.

In the ischemia group; serum I-FABP levels directly correlated with the time of ischemia by significantly increasing after 30, 60, and 90 min of intestinal ischemia (Fig. 2). In comparison with pre-operative serum I-FABP levels, remarkable increases were observed statistically at post-operative 30th, 60th, and 90th min, as shown in Table 2 ($p<0.01$). On the other hand, in the control group, no statistically significant changes were

Table 1. Comparison of serum I-FABP levels between ischemia and control groups

	Ischemia Group	Control Group	p-value
	Mean±SD (ng/ml)	Mean±SD (ng/ml)	
Preoperative	0.69±0.19	0.69±1.77	0.962
30th min	1.38±0.31	0.71±0.19	0.004
60th min	2.02±0.49	0.71±0.29	0.001
90th min	4.54±0.92	0.72±0.18	0.001

I-FABP: Intestinal fatty acid-binding protein; SD: Standard deviation.

Table 2. The increases of serum I-FABP levels according to time in the ischemia group

	Serum I-FABP levels	p-value
	Mean±SD (ng/ml)	
Preoperative versus 30th min	0.69±0.19 versus 1.38±0.31	0.003
Preoperative versus 60th min	0.69±0.19 versus 2.02±0.49	0.001
Preoperative versus 90th min	0.69±0.19 versus 4.54±0.92	0.001
30th versus 60th min	1.38±0.31 versus 2.02±0.49	0.005
30th versus 90th min	1.38±0.31 versus 4.54±0.92	0.001
60th versus 90th min	2.02±0.49 versus 4.54±0.92	0.002

I-FABP: Intestinal fatty acid-binding protein; SD: Standard deviation.

observed in serum I-FABP levels at post-operative 30th, 60th, or 90th min ($p>0.05$).

On histopathologic evaluation, there were pathological signs of ischemia with epithelial erosion, coagulative necrosis, and congested vessels in the lamina propria.

DISCUSSION

AMI is a life-threating acute abdominal disease that emerges as a result of sudden insufficiency in the blood circulation of mesenteric vessels. The major etiologies of mesenteric ischemia are mesenteric arterial embolism (50%), mesenteric arterial thrombosis (15 or 25%), mesenteric venous thrombosis (5%), and non-occlusive mesenteric ischemia due to intestinal hypoperfusion (20 or 30%).[20,21]

The mortality of AMI ranges from 60% to 80%, some reports suggested that it has increased incidence during the last few decades.[2–5,22] The most critical step in the management of AMI is to diagnose the patient with mesenteric ischemia before any intestinal infarction develops. Therefore, studies regarding diagnostic examinations in AMI disease have accelerated in the recent years.

Studies have been performed on numerous markers for the early diagnosis of AMI; however, no marker has been found adequately safe and reliable at this time. Markers such as creatinine kinase, myoglobin, and troponin T and I used in detecting cardiac ischemia have led us to believe that a marker could be found that would be used for intestinal ischemia. Numerous parameters have been tested for early diagnosis of AMI including full blood count and white blood cell count, serum phosphate, LDH, amylase, acid phosphatase, alkaline phosphatase, creatine phosphokinase, alpha-glutamate S-transferase, D-lactate, D-dimer, peritoneal pH measurement, nitric oxide, blood and peritoneal potassium levels, and lipase.[10–14,23] The common result of all these studies is that sensitivities and specificities have not yet been placed into practice to help enable early diagnosis for increased survival rates in AMI.

FABPs are 12–15 Kd cytoplasmic proteins and engage in inner-cell buffering as well as carrying long chained fatty acids. There are nine organ-specific isoforms of FABP.[24] I-FABP is found especially in the epithelial cells of the intestinal mucosa. I-FABP represents a significant portion (2%) of the all protein in enterocytes at the end of intestinal villi and is released into the blood circulation as soon as intestinal mucosal damage occurs.[6,25,26]

Due to the specific intestinal expression of I-FABP, some studies have shown that elevation serum I-FABP levels may indicate intestinal epithelial damage such as mesenter ischemia, strangulated obstruction of the small bowel, necrotizing enterocolitis, celiac disease, ulcerative colitis, abdominal injury, and hemorrhagic shock.[17–19,26–29] In patients with hemorrhagic shock or multiorgan trauma, clinical symptoms in terms of abdominal injury may not be very evident most of the time. In these patients, high I-FABP values can help to demonstrate intestinal injury such as perforation or ischemia. In another case, in strangulated inguinal hernia or umbilical hernia, intestinal ischemia can be detected with high I-FABP values and the surgical strategy can be determined according to the ischemia situation. In such cases, early diagnosis of ischemia provides a chance for early treatment; in this way, it is possible to decrease morbidity and to affect the prognosis positively.

Many studies evaluated that I-FABP could be used in detection of acute ischemic intestinal disease.[17,30–34] In according to the studies which investigating the value of different plasma biomarkers for early diagnosis of AMI, I-FABP is the most promising biomarker for the detection of intestinal ischemia.[35,36] In a bovine experimental study performed by Niewold et al.,[15] the mesentery artery was clamped and intestinal ischemia developed. Consequently, it was detected that I-FABP rose in 15–30 min and advocated using I-FABP in diagnosing mesentery ischemia.

In a study by Cronk et al.,[37] they stated that the sensitivity and specificity of urine I-FABP values were 100% and 83%; while the sensitivity and specificity of serum I-FABP values were 100% and 78%, respectively. According to these results, it was detected that I-FABP is a screening tool that could be used for diagnosis of mechanical intestinal obstruction related intestinal ischemia.

In a multi-center study by Kanda et al.,[30] intestinal ischemia was detected in 52 of 361 patients who were operated on with a pre-operative diagnosis of acute abdominal disorder. They found that the mean serum I-FABP level in the patients with small bowel ischemia was significantly higher than in patients with non-ischemic small bowel disease. Consequently, they have indicated that the measurement of serum I-FABP level is a non-invasive method that is useful for the effective identification of patients with acute abdomen who are at risk for small bowel ischemia.

In our experimental study with rats, the model of AMI was created by SMA ligating in the ischemia group. At the 30th min measurements, it was seen that serum I-FABP values increased significantly in the ischemia group and these values risen progressively over time. In contrast, there was no increase in serum I-FABP values over time in the control group who underwent laparotomy.

One of the limitations of this study is the small sample size. The reason is that our Animal Care and Ethics Committee permitted ten rats only in each group. The second limitation of the study is that our ischemia model only mimicked mesenteric arterial obstruction. There may be different results in

other etiologies of mesenteric ischemia. The last limitation is that we only evaluated serum I-FABP levels; there was no comparison with other markers in the literature for diagnosing AMI. The use of I-FABP as biomarkers needs to be validated in critically ill patients and those who present emergency clinic with acute abdomen.

Conclusion

Early diagnosis of AMI, which has high mortality rates, is life-saving. In our animal model of AMI, serum I-FABP values have increased significantly in the ischemia group and these values have risen progressively over time. We believe that, serum I-FABP may be a useful and promising biomarker for early diagnosis of AMI. We also believe that our results should be supported by clinical studies involving larger patient numbers.

Ethics Committee Approval: All experimental protocols were approved by the Yeditepe University Experiment Animals Ethics Committee (Ref. no: 2011-164).

Peer-review: Internally peer-reviewed.

Authorship Contributions: Concept: S.Z., I.D.P., A.Ç.; Design: S.Z., I.D.P., A.Ç.; Supervision: S.Z., T.Y.; Materials: S.Z., I.D.P., A.Ç., M.Ş., N.B.; Data: S.Z., I.D.P., Y.Ö., D.F., M.M.A.; Analysis: S.Z., I.D.P., Y.Ö., D.F., M.M.A.; Literature search: S.Z., Y.Ö., D.F., M.Ş., M.M.A., N.B.; Writing: S.Z., I.D.P., M.Ş., T.Y.; Critical revision: S.Z., A.Ç., N.B., T.Y.

Conflict of Interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZET*

Akut mezenter iskeminin erken tanısında serum intestinal yağ asidi bağlayıcı proteininin önemi

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AMAÇ: Akut mezenterik iskemi (AMİ), göreceli olarak nadir, ancak sıklıkla erken tanı konulamaması nedeniyle yüksek mortaliteye (%60–80) sahip olan acil bir durumdur. Bu deneysel çalışmadaki amacımız, superior mezenterik arteri (SMA) bağlayarak akut mezenter iskemi modeli oluşturduğumuz sıçanlarda, serum intestinal yağ asidi bağlayıcı protein (I-FABP) düzeylerinde zamanla oluşan değişiklikleri belirlemek ve kontrol grubundaki serum I-FABP düzeyleri ile karşılaştırmaktır.

GEREÇ VE YÖNTEM: Yirmi sıçan, randomize şekilde iki gruba ayırıldı. Kontrol ve iskemi grubu. Bazal serum I-FABP düzeyleri belirlendi. Tüm sıçanlara laparotomi uygulandı ve SMA izole edildi. İskemi grubunda SMA bağlandı ve intestinal iskemi oluşturuldu. Her iki gruptaki tüm sıçanlardan serum I-FABP düzeylerini belirlemek için 30., 60. ve 90. dakikalarda kan örnekleri alındı. Kan sonuçları, iki grup arasında ve her grup içinde zamana göre değişiklikler açısından karşılaştırıldı.

BULGULAR: Mezenter iskemi grubunda, 30., 60. ve 90. dakikalardaki serum I-FABP düzeyleri kontrol grubuna göre anlamlı olarak yüksekti ($p<0.01$). Ameliyat sonrası 30., 60. ve 90. dakikalardaki serum I-FABP düzeyleri ile ameliyat öncesi düzeyler karşılaştırıldığında iskemi grubunda anlamlı yükseklikler saptanırken ($p<0.01$), kontrol grubunda değişiklik olmadığı görüldü. İskemi grubundaki serum I-FABP düzeylerindeki yükselişler iskeminin zamanı ile doğrudan korele idi.

TARTIŞMA: Serum I-FABP düzeyleri, intestinal iskemide anlamlı derecede artmıştır ve bu düzeyler iskemi zamanı ilerledikçe daha fazla yükselmiştir. Serum I-FABP, akut mezenter iskeminin erken tanısı için yararlı ve umut verici bir biyomarker olabilir.

Anahtar sözcükler: Akut mezenter iskemi; biyomarker; erken tanı; I-FABP.

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