The Diagnostic Value of Ischemia-modified Albumin in a Rat Model of Acute Mesenteric Ischemia

Mecit Uygun, MD, Serkan Yılmaz, MD, Murat Pekdemir, MD, Can Duman, MD, and Yeşim Saliha Gürbüz, MD

Abstract

Objectives: Previous studies have demonstrated that ischemia-modified albumin (IMA) is a useful marker for the diagnosis of ischemic events. This study aimed to determine the value of ischemia-modified plasma albumin in the early diagnosis of acute mesenteric ischemia in an experimental model.

Methods: The study was performed on 32 Wistar albino rats divided into control (n = 8), sham (n = 8), 2-hour (n = 8), and 6-hour (n = 8) ischemia groups. Mesenteric ischemia was created by arterial occlusion, and then blood samples (2 mL) were collected and centrifuged. Serum levels of IMA were measured by a rapid calorimetric test that determined the reduced cobalt binding to albumin. For histopathologic evaluation, samples of the small intestine were obtained from the animals after they were euthanized at the end of the experiment.

Results: Histopathologic damage of the intestinal wall correlated with the duration of ischemia. While the mean pathology scores of the 2- and 6-hour ischemia groups were different from each other, IMA levels (mean ± SD) in the four groups were not significantly different from each other: 0.55 ± 0.07 absorbance units (ABSU) in the control group, 0.62 ± 0.09 ABSU in the sham group, 0.60 ± 0.07 ABSU in the 2-hour ischemia group, and 0.64 ± 0.12 ABSU in the 6-hour ischemia group (p = 0.153).

Conclusions: Serum IMA values were not useful in the early diagnosis of acute mesenteric ischemia. Further studies to investigate ischemic and nonischemic conditions that affect IMA levels are needed.


Acutere mesenteric ischemia is a condition that causes an acute abdomen and carries a poor prognosis; mortality rates were 60% to 100% until the early 1980s.1 More recently, early diagnosis and aggressive therapy have lowered the mortality rate to 50% to 70%.2 The poor prognosis is due to a combination of delayed diagnosis, the presence of accompanying systemic diseases in a majority of the patients, and local and systemic effects of intestine ischemia. In an effort to make an early diagnosis and reduce mortality, the diagnostic value of multiple laboratory and radiologic studies has been assessed.

As for other ischemic events, serum markers, such as serum lactate and D-dimer, have been studied for their value in making an early diagnosis of acute mesenteric ischemia. Ischemia-modified albumin (IMA) is a relatively recently described marker that measures cobalt binding to albumin and has been suggested as a valuable tool in the early diagnosis of a variety of ischemic conditions, such as acute myocardial ischemia, pulmonary embolism, and peripheral arterial disease.3–5

Doppler ultrasonography, spiral computed tomography, and mesenteric angiography are commonly used imaging modalities for diagnosing mesenteric ischemia.6–8 These can be invasive, costly, viewer-dependent, and time-consuming. Leukocytosis, acidosis, elevated lactate, hyperphosphatemia, and elevated amylase are nonspecific laboratory markers that are sometimes used for this same purpose. If better markers for the early diagnosis of acute mesenteric ischemia can be used, the
number of imaging tests may be decreased. In this study, the aim was to investigate the value of IMA in the early diagnosis of acute mesenteric ischemia in an experimental animal model.

**METHODS**

**Study Design**

This was a laboratory study of a murine model of acute mesenteric ischemia to assess the utility of IMA for diagnosis. The study was conducted at the Experimental Medicine Research and Practice Unit of Kocaeli University. The protocol was approved by the university ethics committee.

**Animal Subjects**

Thirty-two male Wistar albino rats (weighing 200–250 g each) were obtained for the experiment (Experimental Medicine Research and Practice Unit, Kocaeli University). Before the experiment, all the animals were fed standard rat chow and water for 2 weeks under laboratory conditions. In the 12 hours before the study, the animals were given water only. They were then randomly allocated into four groups with eight rats in each (Group 1 = control; Group 2 = sham; Group 3 = 2-hour ischemia; Group 4 = 6-hour ischemia).

**Study Protocol**

Analgesia was achieved by controlled anesthesia with isoflurane induction and intramuscular administration of 10 mg/kg ketamine. The abdominal skin was shaved and cleansed with povidone-iodine before the experiment. In the control group, intestine resection and blood sampling were performed through a 3-cm midline incision. In the sham group, after 3-cm midline incision, the small intestines were palpated for 1 minute, the mesentery artery was dissected, and then the incision was closed. In the other ischemia groups, after a 3-cm midline incision, the small intestines were removed from the abdominal cavity, the ligament of Treitz was severed, the superior mesenteric artery (SMA) was dissected at its origin from the aorta, and then the SMA and its collaterals were ligated with 3-0 silk suture. Ischemia was then achieved by the Megison method, the safest method for the creation of mesenteric ischemia in rats.9 After the procedure, the abdomen was closed with double 3-0 silk sutures in all groups. In all groups, relaparotomy was performed with 2 mg/kg intramuscular ketamine anesthesia. After 180 minutes in the sham group, and in the 2- and 6-hour ischemia groups, the abdomen was opened and 2-mL blood samples were obtained from the inferior vena cava and the right ventricle. Then, in all groups, the small intestine was removed from the abdominal cavity, and a 5-cm segment of the intestine was excised, 2 cm proximal to the ileocecal valve. The intestinal segments were kept in 10% formaldehyde. At the end of the experiment, all the animals were sacrificed with high-dose isoflurane, in accordance with the Helsinki declaration.

**Histopathologic Evaluation.** All tissue samples were fixed in formalin and subjected to routine tissue evaluations. Sections 4 μm thick were obtained from the paraffin blocks and evaluated under light microscopy after hematoxylin-eosin staining by a pathologist blinded to group allocation. The morphologic changes in the mucosa associated with ischemia were evaluated using the scoring system defined by Chiu et al.10 (Table 1).

**IMA Measurement.** The blood samples were allowed to clot for 30–90 minutes in tubes with no preservatives then were centrifuged at 5,000 rpm for 15 minutes. The samples were stored at ~20°C and processed within 3 weeks. IMA levels in the serum were measured with a rapid calorimetric test that determined the reduced cobalt binding to albumin, as described by Bar-or et al.11 This test is based on the reduction of albumin-cobalt binding due to endothelial damage induced by ischemia on the –NH2 terminal of albumin that is responsible for carrying metals such as lead, nickel, and cobalt. To 200 μL of rat serum placed into a glass tube, 50 μL of 0.1% cobalt chloride (CoCl2·6H2O) was added. To obtain sufficient albumin-cobalt binding, after a slight shake of the tube, the solution was allowed to sit for 10 minutes. As a staining agent, 50 μL dithiothreitol (DTT, 1.5 mg/ml H2O) was added and after 2 minutes, with the addition of 1 mL of 0.9% NaCl, the reaction was inhibited. For each sample measured, a colorimetric control sample was prepared by using a sample made of 50 μL of distilled water and 0.5 mg/mL DTT. The colors of the serum cobalt samples with DTT and without DTT were measured at 470 nm with a model UV160U spectrophotometer (Hitachi U-1900, Hitachi High Tec Corp, Tokyo, Japan). Results are expressed as absorbance units (ABSUs). In this method, results over 0.40 ABSU are considered positive for ischemia (low albumin-cobalt binding) and results under this value are considered negative for ischemia (high albumin-cobalt binding). After albumin-cobalt binding, the detection of nonbinding free cobalt results in high values, which thus reflect reduced albumin-cobalt binding. This method has been verified by the use of radioactive 57Co by Bar-or et al.11 The biochemist was blinded to group allocation as was the pathologist.

**Data Analysis**

Statistical analyses were performed with MedCalc 11.2.1 (MedCalc Turkey Software, Ankara, Turkey). Results were expressed as mean ± standard deviation (±SD), median, interquartile range, and percentage. The Kruskal-Wallis test was used for intergroup compari-

---

**Table 1**

<table>
<thead>
<tr>
<th>Histopathologic Scoring of Ischemic Intestinal Injury</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grade 0:</strong> Normal mucosa</td>
</tr>
<tr>
<td><strong>Grade 1:</strong> Mild ischemia in the mucosa (focal desquamation and congestion)</td>
</tr>
<tr>
<td><strong>Grade 2:</strong> Widespread desquamation and congestion in the mucosa</td>
</tr>
<tr>
<td><strong>Grade 3:</strong> Ischemia extending into the muscular mucosa; congested submucosa</td>
</tr>
<tr>
<td><strong>Grade 4:</strong> Severe ischemia in the submucosa, congestion, and marked necrosis</td>
</tr>
</tbody>
</table>
In the presence of differences, paired samples were compared using the Bonferroni-adjusted Mann-Whitney U-test. p-values < 0.05 was considered statistically significant.

RESULTS

The mean (±SD) weight of the animals was 240 (±10) g. The experiment was completed as planned in all animals.

Gross Pathologic Evaluation

No color changes were observed in the small intestines in the control group after the laparotomy or in the sham group after relaparotomy. However, in the 2-hour ischemia group, the small intestine was noted to have a pinkish-bluish color, and in the 6-hour ischemia group a dark blue-purple color.

Histopathologic Evaluation

No ischemic findings were detected in the histopathology samples of the control group. The histopathologic grade of ischemic damage increased in direct correlation with increased ischemia time (Figure 1, Kruskal-Wallis Ht = 26.53, df = 4, p < 0.0001). Intergroup comparisons revealed that while the control and sham group histopathologic scores were not different from each other (p = 0.442), the mean pathology scores of the other groups were statistically significantly different: control versus 2-hour ischemia (p = 0.0002), control versus 6-hour ischemia (p = 0.0002), sham versus 2-hour ischemia (p = 0.0006), sham versus 6-hour ischemia (p = 0.0002), and 2-hour ischemia versus 6-hour ischemia (p = 0.0019).

Biochemical Evaluation

The mean serum IMA values of the groups were 0.55 ± 0.07 ABSU in the control group, 0.62 ± 0.09 ABSU in the sham group, 0.60 ± 0.07 ABSU in the 2-hour ischemia group, and 0.64 ± 0.12 ABSU in the 6-hour ischemia group (p = 0.153, Kruskal-Wallis Ht = 5.26, df = 3, Figure 2).

DISCUSSION

In this model of mesenteric ischemia in rats, no significant differences in IMA levels were found between IMA values of the control, sham, and intestinal ischemia groups. Acute mesenteric ischemia remains a fatal disease. Much of the morbidity and mortality is primarily associated with delays in diagnosis before intestinal tissue death occurs. Although many patients are well-appearing when they present with some degree of abdominal pain, their condition may rapidly worsen to include tachypnea, hypotension, acidosis, and acute abdomen. The most important factor determining the survival rate is establishing diagnosis before peritonitis and intestinal necrosis develop.

In one experimental study, structural changes were observed in the intestinal wall within 10 minutes after the blood supply to the mesentery was cut off. When ischemia begins, the mucosal cells of the intestinal wall are the first structures to be affected, followed by the muscularis mucosa. Serosal cells are the structures most resistant to ischemia. After changes are seen in the serosa, on a gross pathologic level, the intestine may perforate and/or strictures may develop. Following perforation, free radicals and intestinal contents contribute to the creation of peritonitis.

Researchers have focused on seeking laboratory and radiologic imaging methods that have a high specificity and sensitivity for the early diagnosis of acute mesenteric ischemia. While some abnormalities (e.g., leukocytosis, metabolic acidosis, high serum lactate, creatine kinase, and amylase) may commonly be found during the workup of these patients, they are not specific or sensitive enough to make the diagnosis. D-dimer, for example, was found to be sensitive but not specific for making an early diagnosis.12–14

Figure 1. Histopathologic grade of ischemic damage in rat intestines of rats (n = 8 in each group): 1) control, 2) sham operation, 3) 2 hours of intestinal ischemia, and 4) 6 hours of intestinal ischemia.

Figure 2. Serum IMA levels (measured in ABSU) in rats (n = 8 in each group) undergoing no operation (control), sham operation, 2 hours of intestinal ischemia, and 6 hours of intestinal ischemia. ABSU = absorbance units; IMA = ischemia-modified albumin.
Ischemia-modified albumin is a sensitive and specific biochemical marker in the diagnosis of myocardial necrosis. IMA measured with the albumin-cobalt binding test is based on the temporary loss of metal binding capacity of albumin on the –NH$_2$ terminal of albumin that binds metals such as lead, nickel, and cobalt. This occurs in cases of endothelial damage caused by extracellular hypoxia, acidosis, free radical damage, and Na-K pump dysfunction. During acute ischemic conditions, the metal-binding capacity of albumin for transition metals, like copper, nickel, and cobalt, is reduced, generating a metabolic variant of the protein, commonly known as IMA. The IMA test can reliably determine the presence of ischemia before necrosis develops. IMA levels have also been studied in the context of pulmonary embolism and peripheral arterial disease.

Two recent reports found IMA to be valuable in the early diagnosis of intestinal ischemia. The first, a case-control study, found serum IMA levels (measured using the same rapid colorimetric, albumin-cobalt binding test as we used in our study) to be significantly higher (0.26 ± 0.06 ABSU) in patients with mesenteric ischemia than in those of the control group (0.16 ± 0.02 ABSU). However, these values were both lower than the cutoff value for mesenteric ischemia (0.40 ABSU) proposed by Bar-or et al., leading researchers to question its value as an early serum marker of intestinal ischemia. The same group of researchers then studied IMA levels at different time points in a rat model of mesenteric ischemia in a randomized controlled study. In that study, baseline IMA levels were measured and then measured again after the rats underwent 30 minutes, 2 hours, or 6 hours of intestinal ischemia. Rather than intergroup comparisons, IMA levels of each rat after ischemia were compared to its own baseline level. In paired comparison analysis, IMA values were significantly higher after ischemia than before ischemia.

Although no baseline reference values for serum IMA levels in rats have been reported to date, Bar-or et al. considered IMA values over 0.400 ABSU to be abnormal and represent a marker of ischemia. Recently two reports about experimental ischemia models have showed high serum IMA levels. Dundar et al. reported a rabbit model of mesenteric ischemia and found that significantly higher serum IMA levels were present both in the control group and in the mesenteric ischemia group (means of 0.451 to 0.720 ABSU). Unluer et al. also reported high serum IMA levels for time variable different ischemia groups in another rabbit model (means of 0.60 to 0.65 ABSU). Similar to these findings, our results were also above Bar-or’s cutoff level. Values for IMA binding differed in previous human based studies and animal models. The reason for this difference may be the manual measurement of colorimetric IMA by Bar-or et al. and operator differences in other studies. The variation might also arise from methodologic differences. Another possibility may be the differing of rat serum albumin from human serum albumin in its amino acid sequence: the amino acid sequence in the N-terminus of human serum albumin is Asp-Ala-His-Lys; that of rat serum albumin is Glu-Ala-His-Lys.

In our study, multiple comparisons did not find significant differences in IMA levels among the control, sham, and ischemia groups. While necrosis and histopathologic changes affecting the mucularis mucosa occurred in our rats subjected to intestinal ischemia, IMA levels in these rats were not significantly different from controls and sham-operated animals. Thus, in this animal model, IMA was not a reliable early serum marker of intestinal ischemia.

**LIMITATIONS**

In addition to the differences between human and animal serum albumin discussed above, some of the variation seen may arise from application of the methods. A non-commercial IMA test was used, which may be less reproducible than the standard commercial assay. Also, ischemia-modified albumin is a new biomarker, and serum IMA levels are also affected by various physiologic changes that cause oxidative stress, such as pregnancy; many comorbid conditions such as malignancy, chronic liver failure, end-stage renal failure, cerebral ischemia, and acute infections have also led to false-positive IMA results. We were not able to control all of the variables that could possibly influence IMA levels. Patients with mesenteric ischemia are usually of advanced age and have comorbid diseases, which reduce the specificity and positive predictive value of the test.

**CONCLUSIONS**

Mesenteric ischemia is a medical emergency with a poor prognosis; efforts to reduce mortality include making the diagnosis before intestinal necrosis and/or peritonitis develop. In this rat model of intestinal ischemia, ischemia-modified albumin levels were not significantly higher in rats undergoing up to 6 hours of ischemia, even when gross pathologic and histopathologic changes were observed. Further studies to investigate ischemic and nonischemic conditions that affect ischemia-modified albumin levels are needed.

**References**


---

**Academic Emergency Medicine News on FACEBOOK (on SAEM’s website)**

Please be sure to regularly frequent and follow many activities of the journal on SAEM’s Facebook. Comments on articles are featured there, as well as journal announcements. Another way to keep up to date with the latest information relevant to Academic Emergency Medicine, as well as other emergency medicine topics, happenings, etc!