

Original Research Article

Intestinal fatty acid binding protein (I-FABP) as a marker for acute intestinal ischemia

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ABSTRACT

Background: Acute mesenteric ischemia accounts for around 0.1% of all hospital admission and less than 1% of emergency admissions. However, the presentation is varied, and diagnosis is obscure. The diagnosis of intestinal ischemia demands a high index of suspicion. The overall mortality associated with mesenteric ischemia is between 60% and 93% but rises acutely once bowel wall infarction has occurred. Early diagnosis of ischemia remains the key to reduce morbidity and mortality. I-FABP is a cytosolic protein present in epithelial cells of intestines and released upon ischemia or necrosis of bowel. Author estimated the levels of I-FABP in serum and established it as a marker for acute intestinal ischemia.

Methods: 40 patients admitted to JSS Hospital with suspected acute intestinal ischemia were included in the study. Plasma concentrations were quantified using ELISA for fatty acid binding protein 2. Statistical methods were applied and sensitivity and specificity of serum I-FABP were determined. Confirmation of ischemia was by histopathological analysis of the resected bowel specimen.

Results: 23 patients out of the 40 were diagnosed to have intestinal ischemia. Mean values of serum IFABP levels were significantly higher in patients diagnosed with intestinal ischemia (65.94pg/ml in the non-ischemic group vs 673.53pg/ml in the ischemic group P=0.0002). Cut off chosen in diagnosing intestinal ischemia in this study was 187.59pg/ml. Sensitivity was 95.7% and specificity 88%.

Conclusions: Serum I-FABP can be used for the diagnosis of intestinal ischemia. It is specific and sensitive marker to detect early bowel ischemia.

Keywords: Acute mesenteric ischemia, Bowel ischemia, I-FABP

INTRODUCTION

Acute Mesenteric ischemia accounts for around 0.1% of all hospital admission and less than 1% of emergency admissions. However, the presentation is varied and diagnosis is obscure.

The diagnosis of intestinal ischemia requires a high index of suspicion. The overall mortality associated with mesenteric ischemia is between 60% and 93% but rises acutely once bowel wall infarction has occurred.

Mortality remains greatest for acute mesenteric ischemia resulting from obstruction or embolic phenomena. Patients with an early manifestation of Non-Occlusive Mesenteric Ischemia (NOMI) have mortality rates of 50%-55%, whereas patients with mesenteric vein thrombosis have 15% mortality at 30 days.

The absence of specific biochemical markers and vague clinical signs makes the diagnosis delayed and increases mortality and morbidity of such patients. Intestinal Fatty Acid Binding Protein (I-FABP) is a cytosolic protein

released upon loss of enterocyte membrane integrity in bowel wall ischemia. Timely confirmative diagnosis using this marker will help us intervene earlier and save lives, similar to Troponin I and CKMB in cases of myocardial infarction. The aims and objectives of this study were to determine the sensitivity and specificity of I-FABP as a biochemical marker for intestinal ischemia and to correlate the levels of I-FABP with intestinal ischemia.

METHODS

Forty patients admitted to JSS Hospital, Mysore, India between October 2011 to October 2013 with suspicions of acute intestinal ischemia were included in the study. Plasma concentrations were measured using enzyme-linked immunosorbent assay kit for fatty acid binding protein 2, intestinal FABP2 (Uscn Life Science Inc.) Kit code E90559H. EDTA plasma samples were collected from suspected patients, centrifuged and stored at -80°C. Samples were collected within 1hour of suspicion of ischemia. The detection cutoff for plasma I-FABP was 31.5pg/ml.

Blood was collected in EDTA tubes and centrifuged for 20minutes at approximately 1000×g. Freshly prepared plasma stored in deep freezer at -80°C for analysis. Repeated freeze/thaw cycles were avoided.

Routine Blood investigations CBC, blood urea, creatinine, serum electrolytes was done, serum amylase and lipase were sent to rule out pancreatitis and to determine any correlation. Serum lactate value was determined as it was the only available marker for intestinal ischemia and its levels were correlated with that of IFABP. Final Diagnosis was based upon findings at surgery and histopathological examination of the intestine specimen.

Statistical analysis was done with SPSS V21 for Mac OS by the application of Chi-square tests for association of qualitative variables, t-test was used for continuous variables, Mann Whitney U test was employed for non-parametric variables, descriptive statistics for baseline parameters and Inferential statistics for the test per se. Sensitivity, specificity, Negative Predictive value (NPV), Positive Predictive value (PPV) were calculated by the ROC curve and standard formulas for the same. The patients presenting with acute abdominal pain and suspicion of bowel ischemia were included and the patients with pre-existing intestinal damage due to intestinal surgery within 7 days, other proven causes of acute abdomen-acute appendicitis, hollow viscous perforation, ureteric colic, ulcerative colitis, chronic mesenteric ischemia were excluded.

RESULTS

A total of 40 patients were analyzed in this study. Twenty three of 40 patients had some form of bowel ischemia and

17 patients author diagnosed to have no ischemia. Of the 40 patients enrolled in this study, 12 patients were females (30%) and 28 were males (70%). The patients were predominantly between 21-40years with a mean age of 52years for ischemia and 37years for non-ischemic group. Both age and sex had no statistical significance in this study (Table 1).

Table 1: Age and sex distribution of patients.

Age and sex	Ischemia		P
	Yes (n=23)	No (n=17)	
Sex			
Male	14	14	0.143
Female	9	3	
Age	52.04 (10d-85)	37.35 (20-56)	0.967
0-20	1	1	
21-40	7	10	
41-60	4	6	
61-80	9	0	
81-100	2	0	

Pain abdomen was the predominant symptom on presentation with a mean duration of 3.53days in the ischemic patients and 5.96days in the non-ischemic patients. Malena was present in all the 23 patients in the ischemic group. Additional symptoms are described in Table 2.

Table 2: Symptom analysis of the cohort.

Symptom	Ischemia		P
	Yes (n=23)	No (n=17)	
Distension of abdomen			
0-5	20	17	0.165
6-10	3	0	
Fever			0.9
Present	4	5	
Absent	19	12	
Malena			0.002
Present	23	2	
Absent	0	17	
Guarding			0.2
Present	17	11	
Absent	6	6	
Rigidity			1
Present	11	3	
Absent	11	14	
Radiological diagnosis			0.886
Positive	5		
Negative	18		

Total Leucocyte count was elevated in both the groups with a mean of 12565.22 in patients with ischemia and 11858.82 in non-ischemic patients. Serum Amylase and lipase were elevated in the non-ischemic group with a

mean of 168.18U/L and 155.59U/L. In the ischemic group their respective values were 74.59U/L and 47.59U/L respectively. None of these values had statistical significance (Table 3).

Table 3: Biochemical values of patients with and without ischemia.

Biochemical parameters	Ischemia		P
	Yes	No	
TLC (cells/mm ³)	12565.22	11858.82	0.525
Amylase (U/L)	74.59 (25-262)	168.18 (27-780)	0.073
Liapse (U/L)	47.59 (10-139)	155.59 (15-930)	0.976
Serum lactate (mg/dL)	34.933 (14.01-85)	23.4182 (11.98-36.2)	0.015
Serum I-FABP (pg/mL)	673.53 (136.85-2000)	65.9415 (0-253.78)	0.0002

Serum lactate already a proven marker for intestinal ischemia was found to be elevated in this ischemic group with a mean of 34.93mg/dl and 23.41mg/dl in the non-ischemic group with a significant P value of 0.015. The sensitivity of serum lactate was 87% and specificity was 41%. Serum Intestinal Fatty Acid Binding Protein (IFABP) was significantly elevated in the ischemic group with a mean of 673.53pg/ml versus 65.94pg/ml in the non-ischemic group with a P value of 0.0002 (Figure 1).

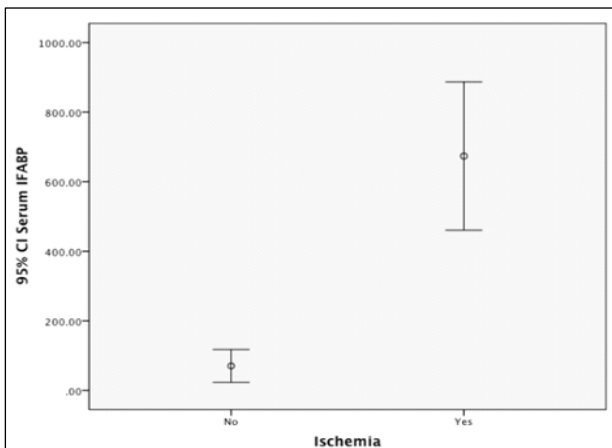


Figure 1: Distribution of I-FABP levels.

Area under the ROC curve was 98% (Figure 2) with a cut off of 187.59pg/ml in this study. The sensitivity and specificity were 95.7% and 88% respectively with a positive predictive value of 91.67% and negative predictive value of 93.75%.

The minimum length of bowel resected was 10cm while the maximum was 1m with a mean of 128.66cm. There was no correlation between length of the bowel resected and serum IFABP levels (P=0.837, R²=0.002). The segment of bowel involved could not be determined prior to laparotomy.

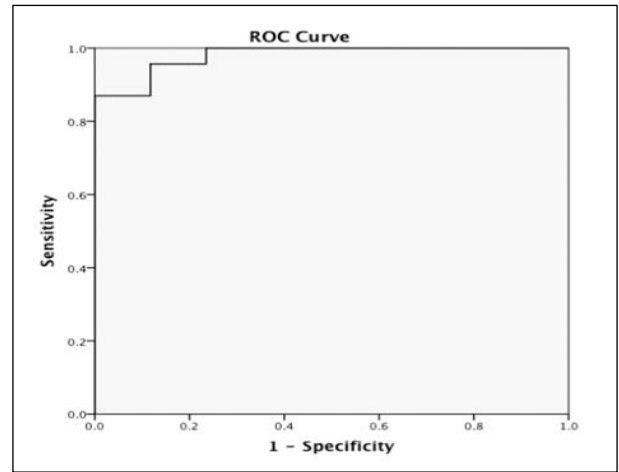


Figure 2: ROC curve for serum I-FABP.

In this study of the 23 patients, diagnosed with ischemia 22 patients were operated. One patient had evidence of ischemia radiologically and was treated with anticoagulants and discharged. Exploratory Laparotomy was performed in 22 patients, ischemic or gangrenous segment of bowel (Figure 3) was resected and sent for histopathological analysis. All histopathological analysis was consistent with findings of ischemia or gangrene.



Figure 3: Intraoperative image of gangrenous bowel.

There were 2 patients had ischemia of the jejunum (8.7%), ileum in 13 patients (56.5%), jejunum and ileum in 2 patients (8.7%), colon in 2 patients (8.7%), colon and ileum in 2 patients (8.7%) and jejunum, ileum and colon in 1 patient (4.4%). Mortality in the ischemic group was 5 patients (21.7%) and non-ischemic group was 1 patient (5.9%).

DISCUSSION

Antonio Benivene of Florence, Italy gave the first description of Mesenteric Ischemia in the 15th century. A scholar of Greek medicine and a busy medical practitioner, he took notes on various gastrointestinal ailments, including gallstones, inflammatory bowel disease and mesenteric venous thrombosis (MVT). His brother discovered these concise notes after Benivene's

death, and they were subsequently published. Centuries later, the intestinal resection and re-anastomosis was employed in treatment of first case of AMI by Elliott in 1895. Goodman reported chronic intestinal angina as one of the clinical disorders in 1918.

In 1926, Cokkinis commented on mesenteric ischemia, "the diagnosis (is) impossible, the prognosis hopeless, and the treatment almost useless"-after reporting a series of 12 deaths caused by mesenteric venous occlusion.

Mesenteric occlusive disease with weight loss and pain out of proportion to abdominal findings leading to death of a patient had been reported in 1936 by Dunphy, a surgical resident at the Peter Bent Brigham Hospital. Studies conducted on the records of 10 such similar cases showed a history of chronic abdominal pain in 7 patients.⁶ Hence, a potential early intervention was needed to prevent disease progression and death.

This study was designed to determine if serum IFABP could be used as a marker to determine ischemia of the bowel. Accordingly, the sensitivity, specificity, negative predictive value and positive predictive value were determined. Plasma lactate, which was most commonly used to determine intestinal ischemia was also evaluated, and its sensitivity and specificity were determined.

Operation

In this study of the 23 patients diagnosed with ischemia 22 patients were operated. One patient had evidence of ischemia radiologically and was treated with anticoagulants and discharged. Exploratory Laparotomy was performed in 22 patients, ischemic or gangrenous segment of bowel was resected and sent for histopathological analysis (Figure 2). All histopathological analysis was consistent with findings of ischemia or gangrene. In the study, conducted by Thuijls et al, patients out of 22 were operated and diagnosed with intestinal ischemia.⁷

Amylase and lipase

In a study conducted by Wilson C et al, serum amylase was determined in 52% of the patients admitted with acute mesenteric infraction.⁸ Amylase levels were normal in 5% and double the normal values in 24%. The amylase levels were found to be in the diagnostic range of acute pancreatitis among five patients (three on admission) leading to inappropriate non-operative treatment in four patients. The findings of this study do not correlate to this study. Serum amylase levels were only useful to eliminate the possibility of pancreatitis as a cause of the presenting abdominal pain in cases with small bowel obstruction and they have no significant value in identifying small bowel viability or perforation. In this study, author concluded that serum amylase can be used to differentiate AMI from acute pancreatitis, which may have a similar clinical presentation.

Serum lactate

Serum lactate refers to the amount of lactic acid in the blood. It increases in conditions of sepsis, septic shock and was a byproduct of anaerobic metabolism (bacterial by-product). Mesenteric ischemia/bowel ischemia results in sepsis elevating levels of serum lactate. It is a late manifestation of bowel ischemia when gangrene sets in and has been the best available marker for intestinal ischemia till date.

In a study conducted by Ozden et al, he concluded that serum lactate is an established marker of cell hypoxia having a sensitivity of 96% but unfortunately it is raised very late in the disease.¹⁰

In a study conducted by Murray et al, reported remarkable elevations in D (-)-lactate levels in patients diagnosed with mesenteric ischemia in comparison with the controls ($P < 50\mu$).¹¹ The sensitivity and specificity of D (-)-lactate level estimations were 90% and 87% respectively with the negative and positive predictive values being respectively at 96% and 70%. The study failed to identify any serum marker that can consistently help in diagnosing acute mesenteric ischemia at its early stages.

Dayton et al, in their article questioned the clinical usefulness of serum lactate.⁹ They mentioned that most of the patients necessitating bowel resection due to ischemia in the setting of small bowel obstruction had normal levels of serum lactate. They further remarked that elevated serum lactate observed during admission may be associated with volume-related global hypoperfusion.

In a study conducted by Lange et al, he concluded that serum lactate was the best available marker to determine intestinal ischemia at that point of time.¹² The sensitivity of his study was 100% with a specificity of 42%, similar to this study. An elevated plasma lactate concentration is always an indicator of an acute life-threatening condition, that usually refers to the need for an emergency operation.

Serum lactate was estimated in 100% of the subjects in the ischemic group. In the group with bowel ischemia, the minimum value of serum lactate was 14.01mg/dl and a maximum of 85mg/dl with a mean of 34.93 ($P=0.015$). The normal values of serum lactate in this laboratory was 4.5-20mg/dl. The sensitivity of serum lactate was 87% while specificity was 41%.

Radiology

Delhom E et al, in their article on cross sectional imaging in acute mesenteric ischemia concluded that CT scan has a 90% sensitivity in the diagnosis.¹³ Bowel wall thickness, enhanced abnormalities, intravascular thrombus, portal venous gas, pneumatosis, bowel dilation and ascites are the significant features observed in CT

and ultrasound imaging of AMI. In a meta-analysis by Jan Menke on the diagnostic accuracy of multidetector CT in acute mesenteric ischemia. 619 cases were studied and 142 were positive for ischemia. The analysis revealed that pooled sensitivity and specificity were 93.3% (with a 95% confidence interval: 82.8%, 97.6%) and 95.9% (with a 95% confidence interval: 91.2%, 98.2%). It concluded that CT scan has a high sensitivity and can be used as the first line imaging.

Firetto MC et al, did a retrospective analysis of findings that were absent in the original reports of multi-detector CT angiography (MDCTA) in patients with suspected acute bowel ischemia.¹⁵ Most of the subtle findings that were overlooked by the initial MDCTA interpretations account for about 33% of the relevant findings of bowel ischemia.

In this study, imaging comprised of ultrasound examination and CT scan. Doppler study, however, could not be performed due to non-availability of well-trained staff during emergency hours and the presence of bowel gas. Out of 23 patients diagnosed with intestinal ischemia only 5 patients (23%) could be diagnosed to have bowel ischemia on imaging. Further as this study comprised of patients having only bowel ischemia in the absence of mesenteric thrombus, 18 patients had bowel dilatation with no other signs of ischemia. Hence, this study was deficient in analyzing the efficiency of imaging in detecting bowel ischemia.

Serum I-FABP

Fatty acid binding proteins are low molecular weight (15-17kDa) cytosolic proteins found exclusively in the epithelial cells. They are a type of cytoplasmic proteins that have the ability to bind long chain fatty acids. These highly tissue specific proteins are amply found in various cell types and perform crucial role in transport, metabolism and intracellular utilization of fatty acids. Nine distinct types of FABP with unique pattern of tissue expression have been identified. Enhanced dynamics due to lower molecular sizes leads to the leakage out of is chemically damaged necrotic cells that in turn resulting in elevated serum levels.

Hence, the absence of FABP facilitates histological characterization of is chemically damaged tissues. Intestinal FABP (I-FABP) that is derived from the human FABP2 gene is found in epithelium cells of the small bowel. Serum levels of I-FABP is one of the potential biochemical markers for intestinal cell damage identification (both in vivo and in vitro) which is undetectable in serum under healthy conditions. In a study conducted by Thuijls GI et al, quantification of plasma and urinary concentrations of liver FABP (L-FABP), intestinal FABP (I-FABP) and ileal bile acid binding protein (I-BABP) were carried out using ELISA. The study was conducted on fifty suspected patients out of whom twenty-two patients were diagnosed with

intestinal ischemia, twenty-four patients were identified with other diseases and the remaining patients were excluded for satisfying exclusion criteria. Median plasma concentrations of both I-FABP, L-FABP and urinary concentrations of all three markers were quantified and identified to be higher in patients with established intestinal ischemia. The estimated plasma I-FABP levels were 653pg/mL vs. 109pg/mL with $P=0.02$.⁷

A study was conducted by Cronk DR et al, for detection of strangulated mechanical small bowel obstruction by using I- FABP. In three patients diagnosed with small bowel necrosis, both urine and plasma I-FABP levels were found to be positive. However, among eighteen patients without necrosis, urine and plasma I-FABP levels were positive in three and four patients respectively. The sensitivity, specificity, PPV and NPV values for urine I-FABP levels are 100%, 83%, 50% and 100% respectively and the same for plasma levels 100%, 78%, 43% and 100% respectively. The study concluded that I-FABP can be used as sensitive marker for ischemia in mechanical small bowel obstruction.

Kanda et al, determined I-FABP levels in a spectrum of 96 subjects that includes 35 healthy subjects, 48 patients hospitalized due to acute abdominal pain and 13 patients diagnosed with ischemic bowel diseases.¹⁷ The studies revealed that serum I-FABP levels (with a cut off 100 ng/ml) can be employed as biochemical marker that help in precise diagnosis of mesenteric infarction. In another study conducted by Kanda et al, serum I-FABP levels were estimated in 361 patients diagnosed with acute abdomen pain.¹⁷ The subjects were chosen from one university and nine city hospitals over more than a year. About 67% of them underwent surgery out of which 52 patients were diagnosed with small bowel ischemia.

The serum I-FABP were determined in all patients with a cutoff level of 3.1ng/ml, and found to be 40.7 ± 117.9 ng/ml in patients diagnosed with small bowel ischemia in contrast with non-ischemic small bowel disease (5.8 ± 15.6 ng/ml) and the same was estimated in patients with diseases not associated with small bowel to be 1.8 ± 1.7 ng/ml. The study deduced that serum I-FABP levels prove to be very efficient biochemical markers in the diagnosis of small bowel ischemia in terms of sensitivity including positive and negative predictive values nevertheless creatinine phosphokinase or lactate dehydrogenase are better in terms of specificity.

In a study by Shi H et al, serum I-FABP levels were estimated in 151 patients admitted with an acute abdomen, 24 of whom were diagnosed with intestinal ischemia.¹⁹ The estimated serum I-FABP levels in patients with intestinal ischemia ((109.67 ± 48.82) µg/L) was considerably higher in comparison with those without intestinal ischemia ((36.78 ± 11.25) µg/L). The cut-off values were fixed at 87.52µg/L for serum I-FABP for the diagnosis of intestinal ischemia. The serum I-FABP concentration of 76.2% was sufficient in terms of

sensitivity and NPV of 96.3% in the diagnosis of intestinal ischemia.

The analysis of serum HIFABP levels from normal individuals and patients with intestinal ischemia was carried out by Lieberman et al, that revealed that the mean levels of HIFABP in various causes of intestinal ischemia: intestinal ischemia-50ng/ml, NEC-14.7ng/ml, systemic inflammatory response syndrome-5.3ng/ml and control-1.87ng/ml.²⁰ These findings suggested HIFABP can assist as a biochemical marker in diagnosing early intestinal mucosal compromise. Edelson et al, studied the levels of plasma I-FABP in neonates diagnosed with necrotizing enterocolitis. I-FABP levels were detected in blood samples of all 7 infants with stage 3 NEC on the other hand, I-FABP levels were detected only in three infants out of 24 infants with stages 1 or 2 NEC. They concluded that serum IFABP can be used as a sensitive marker for NEC. In this study, the sample of one neonate was taken and value of 2000pg/ml was obtained on analysis.

In a study conducted by Sakamoto et al, with a cutoff 7.2ng/ml, for serum I-FABP, it had a sensitivity of 70.0%, specificity of 92.6% and 86.5% in terms of accuracy.²² They also analyzed the relationship between the length of bowel resected and serum IFABP. Linear regression analysis showed a positive correlation between I-FABP levels and lengths of surgically excised bowels ($y=2.527x-7.660$, $r=0.604$, $p=0.0018$). Serum I-FABP sensitively indicates bowel damage in SBO patients and appears to be a potential biomarker for identification of small-bowel ischemia.

Gollin et al, studied the serum levels of IFABP in rats after ligating the mesenteric artery at 30min, 1hour and 3hours followed by reperfusion.²³ Serum IFABP levels rose within 15minutes of ligation of the SMA but serum LDH levels rose only after 3hours. He concluded that IFABP could be used as a sensitive marker to detect early intestinal ischemia.

In this study, IFABP levels were estimated for all 40 patients in this study. In the group with bowel ischemia minimum value of serum IFABP was 136.85pg/ml and maximum were 2000pg/ml with a mean of 673.53pg/ml (Figure 3). Mann Whitney U test was applied to determine the statistical significance of this test P value of 0.0002 (<0.05). 187.59pg/ml was taken as cut off to determine ischemia with a sensitivity of 95.7% and specificity of 88%. Positive predictive value of the test was 91.67%. Negative predictive value of the test was 93.75%. This study did not show any correlation between the length of bowel resected and serum IFABP levels ($p=0.837$, $R^2=0.002$).

CONCLUSION

Intestinal ischemia is a surgical emergency and warrants immediate surgical intervention. Quicker diagnosis aided

by serum I-FABP levels will enable us to intervene in such patients quickly reducing morbidity and mortality among patients. Author concluded that serum I-FABP is a specific, sensitive, quick and cost-effective indicator of intestinal/bowel ischemia. It can identify ischemic changes in bowel at an early stage which will enable us to intervene early and reduce morbidity and mortality. If bowel ischemia is detected earlier, there may be a scope to preserve the bowel by removal of the offending agent, however, this analysis is beyond the scope of present study. The cut off for serum I-FABP levels used in the diagnosis of bowel ischemia in the current study was 187.59pg/ml. The sensitivity of serum I-FABP was 95.7% and specificity was 88%.

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