

RESEARCH ARTICLE

Combination of Serum C-reactive Protein and D-lactate: Predictors of Strangulation in Intestinal Obstruction

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ABSTRACT

Introduction: Serum C-reactive protein (CRP) and D-lactate have been proposed as markers of intestinal ischemia. The purpose of this prospective observational study is to find whether serum CRP and serum D-lactate can help in predicting strangulation in cases of acute intestinal obstruction.

Materials and methods: A total of 50 consecutive patients were included in the study after excluding patients of comorbid medical illness and patients with findings other than obstruction or strangulation. The serum values of CRP and D-lactate were measured and compared between obstruction with strangulation and obstruction without strangulation. Receiver operating characteristic (ROC) curve was drawn and cutoff values for the diagnosis of strangulation were calculated along with calculation of sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs).

Results: A total of 14 patients who were diagnosed with strangulated bowel obstruction had significantly higher values of serum CRP and D-lactate when compared with 36 patients with simple bowel obstruction (116 vs 40 mg/L and 5.4 vs 2.7 mmol/L respectively, $p < 0.05$). Using ROC analysis, the area under the curve of serum CRP and D-lactate was found to be 0.785 and 0.775 respectively, for bowel strangulation. Using a cutoff value of 60 mg/L for CRP, the sensitivity, specificity, PPV, and NPV were 87.5, 58.3, 48.3, 91.3% respectively. Using a cutoff value of 4.5 mmol/L for D-lactate, the sensitivity, specificity, PPV, and NPV were 75.0, 69.4, 52.7, 86.2% respectively.

Conclusion: Serum CRP and D-lactate can be useful and reasonable markers for predicting strangulation in cases of acute intestinal obstruction in an emergency setting.

Keywords: Celiotomy, C-reactive protein, D-lactate, Intestine, Ischemia, L-lactate, Markers.

How to cite this article: Pal AK, Ansari MM, Islam N. Combination of Serum C-reactive Protein and D-lactate: Predictors of Strangulation in Intestinal Obstruction. *Panam J Trauma Crit Care Emerg Surg* 2016;5(3):134-139.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Intestinal obstruction is one of the commonest clinical problems encountered in surgical practice. One of the grave complications of intestinal obstruction is strangulation, which requires an emergency celiotomy for management. Ischemia, which complicates 7 to 42% of bowel obstructions, significantly increases mortality associated with bowel obstruction.¹ Time is an essence, with an earlier diagnosis of strangulation favoring increased patient's survival. The diagnosis of strangulation is primarily clinical, with a sudden onset of pain, i.e., continuous rather than colicky, the early appearance of shock, and the presence of fever, tachycardia, marked abdominal tenderness, guarding, rebound tenderness, and a tender abdominal mass are all in favor of the diagnosis of strangulation.¹ Various biochemical markers, such as values of serum tumor necrosis factor α , C-reactive protein (CRP), interleukin 6, D-lactate, D-dimer, alpha glutathione S-transferase, intestinal fatty acid binding protein (I-FABP), creatine kinase B, isoenzymes of lactate dehydrogenase, procalcitonin, alkaline liver phosphatase, and urinary phosphate have been studied²⁻²⁵ in cases of intestinal obstruction and/or intestinal ischemia/strangulation mainly in animal models or tissues with few in clinical settings, with relatively few studies in humans on finding a biomarker of preoperative strangulation. Therefore, studies investigating the role of biomarker in predicting strangulation in patients of acute bowel obstruction are needed.

C-reactive protein is a protein found in the blood, the levels of which rise in response to inflammation (an acute-phase protein). The CRP is synthesized by the liver in response to factors secreted by adipocytes.²⁶ It is a member of the pentraxin family of proteins.²⁶ Rapid, marked increases in CRP occur with inflammation, infection, trauma and tissue necrosis, malignancies, and autoimmune disorders.²⁶ Lactic acid is the normal endpoint of the anaerobic breakdown of glucose in the tissues. Lactic acid exists in two optical isomeric forms, L-lactate and D-lactate. D-lactate is not produced in mammalian tissue, but it is detected in a situation of an abnormal proliferation of enteral bacterial flora due to mucosal injury following mesenteric ischemia.¹⁴⁻¹⁶ The aim of this

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prospective observational study was to evaluate role of CRP and D-Lactate in combination as markers of bowel strangulation.

MATERIALS AND METHODS

Patients

A total of 50 consecutive adult patients presenting to the emergency complex of Jawaharlal Nehru Medical College, Aligarh, India, between February 2010 and November 2011 with clinical suspicion of acute intestinal obstruction and operated in the emergency operation theater were included in the study. Various intraoperative findings causing acute intestinal obstruction led to formation of two groups, i.e., simple bowel obstruction and strangulated bowel obstruction. Patients with coexisting medical illnesses, such as chronic kidney diseases, any cardiac ailment, diabetes mellitus, and coagulopathy were excluded from the study, since these illnesses can alter the course of obstruction strangulation sequence in patients with acute intestinal obstruction. They can also lead to false-positive results, especially diabetes and chronic kidney disease. In addition, patients with any intraoperative findings apart from simple or strangulated bowel obstruction were excluded. Demographic characteristics of age, gender, and clinical findings of duration of symptoms, diffuse pain, generalized distension of abdomen, vomiting, fever, obstipation, history of previous abdominal surgery, shock (systolic blood pressure <90 mm Hg), tenderness, guarding or rigidity, and exaggeration of bowel sounds were taken for study. The study was ethically approved by the Institutional Ethics Committee, and informed consent was taken from all patients. There was no financial support from any source for this study.

Laboratory Tests

Blood samples (venous) from 83 patients were taken at the time of presentation in the emergency within 20 minutes of their arrival, and all had suspicion of acute intestinal obstruction.

Blood was collected and sent to the Department of Biochemistry for separation of sera by centrifugation and storage in sterile vials at -20°C and, thereafter, measurement of total leukocyte count (TLC), serum CRP levels, and serum D-lactate levels were done.

Measurement of Serum CRP Levels

Quantitative assessment of serum CRP was done using human CRP kit (hs CRP ELISA, 96 wells, OKBA00016, Aviva Systems Biology; <http://www.avivasysbio.com/highsensitivitycreactiveproteinenzymeimmunoassay-testkit96wells.html>) based on the principle of solid-phase enzyme-linked immunosorbent assay. During this

estimation, the assay system utilized a unique mouse anti-CRP monoclonal antibody directed against a distinct antigenic determinant on the CRP molecule. A goat anti-CRP antibody is in the antibody enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CRP molecules being sandwiched between the solid phase and enzyme-linked antibodies. The details of the procedure are described elsewhere by Shine et al.²⁷ and Roberts et al.²⁸ Using this method, values of 0 to 100 ng/mL could be measured and the reference normal range in healthy population was taken as 0.08 to 3.1 ng/mL.^{26,27}

Measurement of D-lactate Levels

D-lactate was measured using the D-lactate assay kit (colorimetric, ab83429; <http://www.abcam.com/d-lactate-assay-kit-colorimetric-ab83429.html>) based on the principle of proportionate color generation as a result of oxidation of D-lactate by D-lactate dehydrogenase. The details of the procedure were described by McLellan et al.²⁹ Using this method, values between 0.01 and 10 mmol/mL could be measured, and the normal serum D-lactate value is in the nano- to micromolar range.²⁹

Statistical Analysis

Data were analyzed in terms of demographics, clinical features, blood tests (white blood cells, serum CRP, and serum D-lactate) at the time of admission and operative findings.

Tables were prepared using either Pearson's chi-square test to compare the clinical characteristics, or operative findings and blood investigations between the patients with strangulation and patients without strangulation. The p-values of <0.05 were considered to be statistically significant.

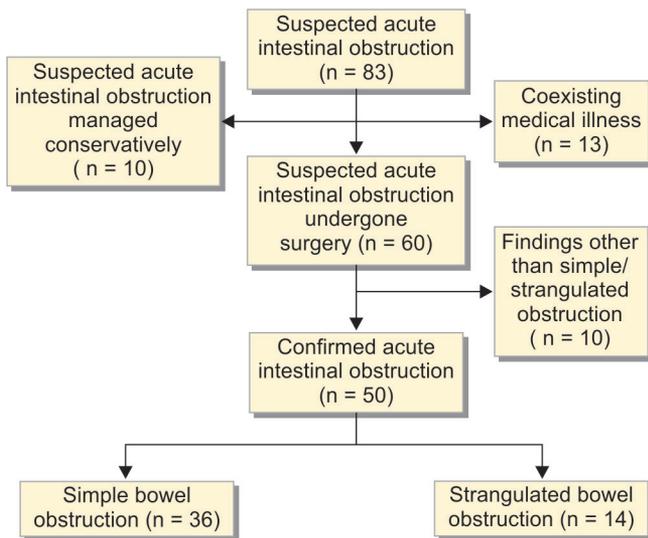
A receiver operating characteristic (ROC) analysis was also performed for serum CRP and D-lactate, and the area under the ROC curve was acquired. All analyses were performed using Statistical Package for the Social Sciences version 17 (IBM).

RESULTS

Patient Characteristics

Eighty-three patients with acute abdomen were suspected as having bowel obstruction (Flow Chart 1). Blood samples of all of these patients were taken for measurement of desired biochemical markers. Ten patients with bowel obstruction, who were managed conservatively, and 13 patients with coexisting medical illness were excluded from the study; 60 patients with bowel obstruction were operated and 10 patients with intraoperative findings other than obstruction and/or strangulation were also

Flow Chart 1: Eighty three patients with acute abdomen were suspected with bowel obstruction. Ten patients with bowel obstruction who were managed conservatively and thirteen patients with coexisting medical illness were excluded from the study and 60 patients with bowel obstruction were operated and ten patients with intraoperative findings other than obstruction and/or strangulation were also excluded and finally fifty patients were included in this study. According to the findings of surgery they were divided into the two groups, those with strangulated bowel obstruction or those with simple bowel obstruction



excluded; finally, 50 patients were included in this study. According to the findings of surgery, they were divided into two groups: Those with simple bowel obstruction or those with strangulated bowel obstruction. There were 33 males in the study (Table 1) with median age of 50 years (28–70 years).

On comparing the clinical findings, presence of exaggerated bowel sounds was significantly higher in simple obstruction ($p < 0.05$). Strangulation was present in 10 out of 35 patients with small bowel obstruction, while 4 out of 15 had strangulation in large bowel obstruction. Another notable finding was that during surgery (Table 2), the common findings were postoperative adhesions and Koch’s abdomen in both of the groups without any ascertainable difference.

Comparison of Laboratory Markers

Among blood investigations, serum values of CRP and D-lactate (Table 3) were significantly higher in patients with strangulated bowel obstruction as compared with simple bowel obstruction (116 vs 40 mg/L and 5.4 vs 2.7 mmol/L respectively, $p < 0.05$). Although nonsignificant ($p > 0.05$), the values of TLC were also higher in the strangulated bowel obstruction (10,500 vs 16,800 per μ L). Using ROC analysis (Graph 1), the area under the curve of serum CRP and D-lactate was found to be 0.785 and 0.775 respectively, for bowel strangulation. For predicting

Table 1: Patient characteristics

Patient characteristics	Simple BO (n = 36)	Strangulated BO (n = 14)	Total (n = 50)
Age (median, year)	48	54	50
Gender (M, n)	22	11	33
Duration of symptoms (median, hours)	28	26	24
Diffuse pain (%)	30 (83)	11 (79)	41 (82)
Generalized distension (%)	30 (83)	10 (71)	40 (80)
Vomiting (%)	30 (83)	4 (29)	34 (68)
Fever (%)	2 (5)	10 (71)	12 (24)
Obstipation (%)	25 (69)	13 (93)	38 (76)
History of previous abdominal surgery (%)	20 (56)	4 (29)	24 (48)
Shock (%)	10 (27)	12 (86)	22 (44)
Tenderness (%)	23 (64)	10 (71)	33 (66)
Guarding/rigidity (%)	14 (39)	7 (50)	21 (42)
Exaggeration of bowel sounds (%)	33 (92)	11 (79)*	44 (88)

* $p < 0.05$, significant; BO: Bowel obstruction

Table 2: Comparison of the operative findings

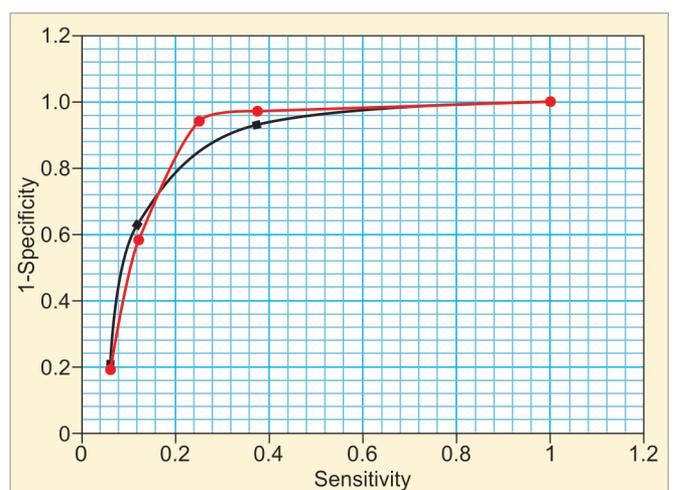
Cause of obstruction	Simple BO (n = 36)	Strangulated BO (n = 14)	Total (n = 50)
Postoperative adhesions (%)	16 (44)	4 (29)	20 (40)
Tuberculosis (%)	16 (44)	4 (29)	20 (40)
Hernia (%)	2 (6)	2 (14)	4 (8)
Bands (%)	1 (3)	0 (0)	1 (2)
Neoplasms (%)	1 (3)	1 (7)	2 (4)
Volvulus (%)	0 (0)	3 (21)	3 (6)

BO: Bowel obstruction

Table 3: Blood investigations

Laboratory tests	Simple BO (n = 36)	Strangulated BO (n = 14)	p-value
Total leukocyte count	10,500	16,800	0.21
Serum CRP (mg/L)	40.0	116.0	0.04*
Serum D-lactate (mmol/L)	2.7	5.4	0.03*

BO: Bowel obstruction; * $p < 0.05$, significant



Graph 1: Receiver operating characteristics curves showing diagnostic performance for strangulated bowel obstruction. Areas under the curve (95% confidence intervals) for each marker are as follows: C-Reactive Protein(CRP), 0.785 (0.322–0.934); D-lactate, 0.775 (0.331–0.898). The cut-off levels of CRP and D- Lactate concentration at which the Youden index exhibited a maximum value were 60 mg/L and 4.5 mmol/L respectively



Table 4: Comparison of the diagnostic capability of the studied biochemical markers for predicting strangulated bowel obstruction

Markers	Areas under the ROC curve	Cutoff values	Sensitivity	Specificity	PPVs	NPVs
CRP	0.785	60.0	87.5	58.3	48.3	91.3
D-lactate	0.775	4.5	75.0	69.4	52.2	86.2

strangulation, using a cutoff value of 60 mg/L for CRP, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 87.5, 58.3, 48.3, and 91.3% respectively (Table 4), and using a cutoff value of 4.5 mmol/L for D-lactate, the sensitivity, specificity, PPV, and NPV were 75.0, 69.4, 52.7, and 86.2% respectively (Table 4).

DISCUSSION

Strangulation is a complication associated with acute intestinal obstruction and requires prompt diagnosis. This is easier said than done, especially in an emergency setting. Moreover, reported mortality rates after acute intestinal obstruction have found association with delay in surgical management with progression to strangulation in many cases.¹

Our study has shown that serum CRP and serum D-lactate levels were significantly raised in strangulated bowel obstruction as compared with simple bowel obstruction.

With regard to serum CRP, various animal and human studies have shown that it is raised in mesenteric ischemia and strangulation. In a study by Demir et al,³⁰ done for identifying factors predicting the need for surgery, elevated CRP was significantly associated with bowel gangrene and an underlying predictive value for the need of surgery. Another study by Lin et al³¹ for evaluation of risk factors for intestinal gangrene identified that elevated serum CRP was associated with bowel gangrene.

Most of the lactate found in human body is L-lactate. Van Noord⁸ studied 49 patients with chronic gastrointestinal ischemia and found that L-lactate elevation was significantly increased in ischemia as compared with the nonischemia group.

Serum D-lactate, another marker used in our study, is produced in the human body mainly by the glycoylase pathway, and the normal serum value is in the nano- to micromolar range; thus, the elevation of D-lactate is almost always pathological with strong association found in short bowel syndrome and intestinal ischemia/strangulation.¹⁴⁻¹⁶ Markogiannakis et al¹ had also reported finding in favor of serum lactate as predictor of ischemia and strangulation. D-lactate, specifically, was studied by Murray et al¹² in a prospective study in patients with acute abdominal emergency including mesenteric ischemia. They concluded that serum D-lactate can help in diagnosing patients with

acute mesenteric ischemia. Poeze et al³² found that serum D-lactate is consistently increased in intestinal ischemia, with sensitivity and specificity of 82 and 72% respectively. Evennett et al³³ have also added to this point of view.

Recently, a retrospective study³⁴ has shown that increase in I-FABP levels in patients with small bowel obstruction can be used to predict strangulation. The I-FABP can be increased with dynamic recycling of enterocytes during ischemia or strangulation, but I-FABP measurement requires a long period of time, while measurement of serum CRP and serum D-lactate can be done in a short span of time. Another study evaluating procalcitonin³⁵ as a marker for strangulation in cases of acute small bowel obstruction found it to have a PPV of 95% and an NPV of 90%. However, the results of this study should be taken into account along with the possibility of bacterial translocation occurring in cases of intestinal obstruction without strangulation resulting in false elevation of procalcitonin.

With regard to operative findings in our study, postoperative adhesions and abdominal tuberculosis were the two most common findings reaffirming the high incidence of abdominal tuberculosis in this part of the world. Besides, the incidence of strangulation was the highest in cases of volvulus as all three patients had strangulated bowel obstruction entailing the need of urgent surgical intervention in these patients with minimal delay.

The main strength of our study lies in the fact that it is a prospective study with applicability in an emergency setting in a developing country, where availability of computed tomography (CT) and other costly biomarkers is beyond the reach of poor patients. Also, the patients in our study included those with both small and large bowel obstruction. To the best of our knowledge, this is the first study to evaluate the combination of serum CRP and D-lactate for predicting strangulation in cases of acute intestinal obstruction. However, there are certain limitations to the study as well. First of all, study participants were less in number and patients of pediatric age group were not part of this study. Second, the markers studied were only serum CRP, D-lactate, and TLC, and there was no separation of patients requiring resection of bowel due to strangulation. Another limitation in our study was the time required for analyzing D-lactate that ranged from 6 to 48 hours depending on availability of the D-lactate assay kit in our emergency setting, with each testing costing

about 300 INR (approx 4 USD). Lastly, the radiological findings, postoperative outcome, length of hospital stay, and follow-up were not assessed by markers with regard to diagnosis of strangulation.

For tackling these limitations, a prospective study involving a large number of patients of pediatric, adolescent, and adult age groups for evaluating the role of various biomarkers including serum CRP, D-lactate, procalcitonin, and I-FABP is required in patients with acute intestinal obstruction, and correlation of these markers with radiological findings on X-ray or contrast-enhanced CT abdomen should be sought. Such a study would help in decreasing the time interval to surgery in cases of acute intestinal obstruction with strangulation as well as decreasing unwarranted laparotomy in those cases of intestinal obstruction without strangulation, in which a trial of conservative management seems feasible based on other parameters and clinical findings. Improvements in measuring techniques of D-lactate should be sought to limit measurement time and finding quick results for clinical decision making. These biomarkers can be readily available in the emergency setting after due consideration given to their clinical relevance at the institutional level.

The present study demonstrated that the serum levels of CRP and D-lactate were significantly higher in patients with strangulated acute intestinal obstruction and played a role in discrimination between two groups with NPV of both CRP and D-lactate considerably higher. The reason of a lower PPV could be due to small sample size, and the occurrence of strangulation in the study may not be actually mimicking the prevalence in population.

Nevertheless, this study does add to the current literature regarding the need of decision-making policy for management of acute intestinal obstruction incorporating the role of biochemical markers for predicting ischemia/strangulation at the time of presentation.

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