

# Diagnostic Value of Blood D-dimer Level in Acute Mesenteric Ischaemia in the Rat: An Experimental Study

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**OBJECTIVE:** The high mortality rate of mesenteric ischaemia is mainly due to delay in diagnosis. For this reason, it is of great importance to find a specific and rapidly elevating marker. The present study investigated the diagnostic value of blood D-dimer level as a potential marker for acute mesenteric ischaemia in a rat model.

**METHODS:** Thirty male Wistar albino rats were divided into three groups. Basal D-dimer and L-lactate levels were determined in the non-operative control group (I). In the operated control group (II), the superior mesenteric artery was simply manipulated, while the artery was ligated in Group III. Blood samples were drawn in all groups for D-dimer and L-lactate assays.

**RESULTS:** Both Group II ( $p = 0.016$ ) and Group III ( $p = 0.001$ ) had significantly higher mean D-dimer levels in the first postoperative hour compared with the basal level in Group I. However, there was no difference between the levels in Groups II and III. The mean level in Group II in the sixth hour had dropped to a statistically insignificant level compared with the basal value, while the mean value in Group III kept rising during this period ( $p = 0.001$ ). Nevertheless, there was no significant difference between Groups II and III. On the other hand, the mean L-lactate level in the first postoperative hour in Group III was significantly higher than the basal level in Group I ( $p = 0.003$ ). No significant rises were recorded in Group II in the first and sixth postoperative hours. The difference between Groups II and III in the first hour was significant ( $p = 0.005$ ). Group III also had significantly higher mean serum L-lactate value in the sixth hour compared with both the basal value in Group I ( $p = 0.001$ ) and the sixth-hour value in Group II ( $p = 0.003$ ).

**CONCLUSION:** These results do not adequately support the use of blood D-dimer level as an independent parameter in the diagnosis of mesenteric ischaemia due to arterial thrombosis. However, this parameter can be used together with other tests in eliminating the possibility of a thromboembolic event. [*Asian J Surg* 2005;28(2): 131-5]

**Key Words:** D-dimer, diagnosis, L-lactate, mesenteric ischaemia, rat, superior mesenteric artery

## Introduction

Despite recent advances in surgical technique and perioperative care, the mortality rate of acute mesenteric ischaemia remains very high.<sup>1-3</sup> The only solution for this unacceptably high mortality rate seems to be to diagnose the pathology as

early as possible. Many laboratory parameters such as serum lactate have been assayed for this purpose, but no clinically useful results have been obtained. Most test substances are non-specific and require severe tissue injury in order to appear in the serum. The translation of this into surgical practice is a delay in diagnosis until irreversible bowel damage occurs. For

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this reason, it is of paramount importance to find a specific and rapidly elevating marker to diagnose intestinal ischaemia at an early stage of the disease to give a chance to both the patient and surgeon.

D-dimer is a fibrinolytic marker that has been used in the diagnosis of thromboembolic events.<sup>4</sup> A recent clinical pilot study shows that D-dimer could also be useful in identifying patients with mesenteric ischaemia.<sup>5</sup> In light of this finding, this experimental study investigated the value of blood D-dimer levels in the diagnosis of acute mesenteric ischaemia in a controlled rat model.

## Methods

This study was approved by the local ethics committee of the Ankara Numune Teaching and Research Hospital and conducted in Ankara Etlik Veterinary Research Institute. Blood tests were assayed in the Hematology and Metabolism Laboratories and histopathological examinations in the Department of Pathology of the same hospital.

Thirty male Wistar albino rats weighing 250–300 g were used. All rats were fed standard rat chow until the experiment.

### *Surgical procedure*

Rats were treated under general anaesthesia using 50 mg/kg intramuscular ketamine hydrochloride. Following shaving, surgical site antisepsis was carried out with iodine solution. Subjects were divided into three groups. In Group I (non-operative control,  $n = 10$ ), blood samples were drawn for basal blood D-dimer and serum L-lactate levels but no surgery was done. In Group II (operated control,  $n = 10$ ), no preoperative blood samples were obtained. After midline laparotomy, the small bowel mesentery and superior mesenteric artery (SMA) were simply manipulated. Postoperative blood samples for D-dimer and L-lactate tests were withdrawn at the first and sixth hours. In Group III (ligation,  $n = 10$ ), no preoperative blood samples were obtained. The SMA was ligated with 3/0 silk following a midline laparotomy. Postoperative blood samples for D-dimer and L-lactate assays were withdrawn at the first and sixth hours.

### *Laboratory tests*

Blood (4 mL) collected in a citrated tube was centrifuged at 3,500 rpm (1,300g) for 10 minutes at room temperature for D-dimer assay using latex agglutination (D-dimer Plus commercial kit, Dade Behring BCT, Marburg, Germany). A 1-mL aliquot of each blood sample was transferred to the laboratory

in an ice bag for serum L-lactate level determination using an enzyme-membrane method (2300 STAT Plus system, membrane installation solution 2329/system buffer 2357/calibration standard 2328/linearity check solution 1530/starter kit 2325; Yellow Springs Instruments Inc, Yellow Springs, OH, USA).

### *Histopathological examination*

The small bowel and its mesentery were resected in Groups II and III. Histopathological examination was carried out under light microscopy to evaluate mesenteric ischaemia and major thrombus formation within the SMA.

### *Statistical analysis*

Comparisons of mean D-dimer and L-lactate levels between groups were made using the independent sample *t* test. The differences between first- and sixth-hour measurements in each group were analysed by paired sample *t* test. A *p* value of less than 0.05 was considered significant.

## Results

### *Operative findings*

No mortality was encountered throughout the experiment. Obvious cyanotic changes were observed in the small bowel of all rats in Group III, the ligation group, whereas in Group II, the operated control group, subjects had normal-appearing intestines.

### *Laboratory tests*

The mean blood D-dimer level in Group I was 48.1 mg/L. Both Groups II ( $p = 0.016$ ) and III ( $p = 0.001$ ) had significantly higher mean D-dimer levels in the first postoperative hour, but there was no difference between the values in Groups II and III (Table 1). By the sixth hour, the mean D-dimer level in Group II had dropped to a level that was not significantly different from the basal level in Group I, while in Group III, it continued to rise ( $p = 0.001$ ). No significant difference was observed between Groups II and III.

The mean serum L-lactate level in Group I was 1.67 mmol/L, significantly lower than in Group III at the first postoperative hour ( $p = 0.003$ ) (Table 2). No significant increases were recorded in Group II in either the first or sixth postoperative hours. The difference between Groups II and III in the first hour was significant ( $p = 0.005$ ). Group III also had significantly higher mean serum L-lactate level in the sixth hour compared with both Group I ( $p = 0.001$ ) and Group II ( $p =$

**Table 1.** Blood D-dimer levels (mg/L)

	Basal	1 <sup>st</sup> hour	<i>p</i> *	6 <sup>th</sup> hour	<i>p</i> *
Group I	48.1 ± 2.4	–		–	
Group II		53.3 ± 5.4	0.0016	52.0 ± 7.7	
Group III		55.5 ± 6.1	0.001	57.7 ± 3.0	0.001

\*Versus Group I.

**Table 2.** L-lactate concentration (mmol/L)

	Basal	1 <sup>st</sup> hour	<i>p</i> *	6 <sup>th</sup> hour	<i>p</i> *
Group I	1.67 ± 0.26	–		–	
Group II		1.76 ± 0.30		1.85 ± 0.35	
Group III		3.03 ± 0.98	0.003*, 0.005 <sup>†</sup>	3.36 ± 1.01	0.001*, 0.003 <sup>†</sup>

\*Versus Group I; <sup>†</sup>versus Group II.

0.003) in the sixth hour.

There were no ischaemic findings in the small bowel or major thrombus formation within the SMA in Group II subjects. On the other hand, all Group III subjects had microscopic changes related to ischaemia in the first hour and these involved the entire bowel wall by the sixth hour.

## Discussion

The earliest marker so far in the diagnosis of mesenteric ischaemia has probably been serum phosphate. In a dog model, Jamieson and colleagues have shown that serum phosphate levels rise in massive intestinal necrosis.<sup>6</sup> However, this increase does not occur when a limited segment of the bowel is affected and the serum phosphate level remains high for a short period of time. Similarly, other potential markers such as lactic dehydrogenase, amylase, alkaline phosphatase, creatinine phosphokinase and ammonia were found to have no specificity for mesenteric ischaemia.

It seems that in mesenteric ischaemia, the best of the evaluated markers to date is serum lactate. Several clinical and experimental studies have shown that the serum lactate level increases following superior mesenteric artery occlusion. Newman and colleagues have stated that an elevated serum lactate concentration also predicts mortality in mesenteric infarction cases.<sup>7</sup> Lange and Jackel have reported 100% sensitivity, but only 42% specificity, for serum lactate as a marker for mesenteric ischaemia.<sup>8</sup> In another clinical study, measurement of serum lactate was diagnostically helpful, although not confirmative.<sup>9</sup>

Lactate is simply an indicator of an anaerobic state. This well-known parameter was used in the present study as a control test for a new potential marker, D-dimer. As expected, the mean serum L(+)-lactate concentration in Group III was higher than those in Groups I and II. This increase appeared as early as the first postoperative hour, and the significant difference remained at the sixth hour.

In the present study, D-dimer, which reflects the extent of fibrin turnover, was studied as a possible marker for mesenteric ischaemia. The D-dimer level rises when the coagulation system is activated. It has long been accepted as the most useful laboratory marker in detection of thromboembolism and has a proven role in the diagnosis of venous embolism of the lower limb and pulmonary embolism.<sup>4,10</sup> However, its value in arterial thrombotic events needs further evaluation.<sup>11</sup>

Acosta and colleagues recently reported a clinical study of acute bowel ischaemia.<sup>5</sup> In this pilot study with a limited number of patients suffering from mesenteric ischaemia, six patients who had embolic or thrombotic mesenteric events had significantly higher D-dimer levels than eight other acute abdomen patients with different intra-abdominal pathologies. Their study was prompted by the fact that the fibrinolytic system is activated following acute arterial thrombosis, for example, D-dimer levels are increased in patients with symptomatic abdominal aortic aneurysms.

The first limitation of D-dimer testing in surgical practice is its low specificity in many conditions. Every surgical trauma and tissue injury can activate coagulation and, consequently, the fibrinolytic system.<sup>12-14</sup> In the present study, we designed

two control groups, non-operative (Group I) and operated (Group II), in order to interpret the D-dimer test results in rats with the SMA ligated. This was mandatory because no previous cut-off level was determined in either rat studies or mesenteric ischaemia.

Clinical studies of mesenteric ischaemia have shown that early diagnosis is key to a successful outcome. Therefore, any new marker to be investigated should have significantly increased concentrations immediately following the initiation of the clinical pathology that results in mesenteric ischaemia. D-dimer can be an appropriate marker in that sense, because it is the most rapidly elevating marker among fibrinolytic products: in bleeding oesophageal varices, it increases significantly only 5 minutes after endoscopic embolization with thrombin.<sup>15</sup> The same increase can be anticipated after the occlusion of the SMA, and this may aid in rapid diagnosis and treatment of mesenteric ischaemia.

Any surgical trauma or even intramuscular injection itself may cause elevated D-dimer values. Thus, it was expected that mean D-dimer levels in both operated groups (II and III) in the first postoperative hour were significantly higher than that in the non-operative control group (I). When we compared the two operated groups, SMA-ligated rats had somewhat higher D-dimer levels than the sham-operation group. The anticipated results for the sixth hour of the experiment were a decrease in the D-dimer level in Group II, as the effect of the surgical trauma resolved, and an elevated or at least sustained D-dimer level in Group III due to the continuation of fibrinolytic activation. Observed results were as expected, but the difference between the two groups did not reach significance. These results are supportive of the diagnostic value of D-dimer, but they do not allow a definitive conclusion. Even if D-dimer levels in the SMA ligation group reached a significantly higher level due to ongoing fibrinolytic activation by, for example, the 24<sup>th</sup> hour, this would not allow an early diagnosis. In fact, no cases in Acosta and colleagues' study, in which D-dimer was suggested as a possible marker for occlusion of the SMA, had early presentation and five of the six patients underwent bowel resection; the same number of patients died within hours or days.<sup>5</sup>

Another possible explanation for no significantly higher D-dimer levels after ligation of the SMA compared with operated control subjects is that in arterial thrombotic events, this marker does not increase as much as in venous thrombosis. Lowe and Rumley report that thrombus formation of arterial origin and activation of coagulation and coronary heart diseases can only result in a modest elevation in D-dimer levels.<sup>16</sup>

Microscopic examination of the mesentery of SMA-ligated rats revealed no major venous thrombi, which might be another reason for the lack of significantly elevated D-dimer levels in this group of rats compared with the operated control group.

The main advantage of D-dimer in the diagnosis of thromboembolic events is high negative predictive value.<sup>4</sup> This means that although D-dimer might be elevated in many non-specific conditions, it is certainly elevated in every patient with venous thromboembolism. Therefore, if the result is normal, this precludes the possibility of thromboembolism. Another proposal for more effective use of D-dimer in cases with suspected thromboembolism is the use of pre-test probability, which means that groups should be separated as low probability and high probability according to other features. Kearon et al conducted a study of deep venous thrombosis using a pre-test checklist of nine items.<sup>17</sup> In the low pre-test probability group, they found a negative predictive value of 99.4%. A similar pre-test checklist can be designed for mesenteric ischaemia, including risk factors such as cardiac arrhythmias, advanced age, generalized atherosclerosis, severe valvular disease and history of myocardial infarction.<sup>18</sup>

In conclusion, our results do not adequately support the use of blood D-dimer levels as an independent parameter in the diagnosis of mesenteric ischaemia due to an arterial thromboembolic phenomenon. However, high D-dimer levels may reflect activation of coagulation and fibrinolytic systems. Therefore, measurement of D-dimer levels can be of value in eliminating the possibility of a mesenteric thromboembolic event when the value is in the normal range, and D-dimer assay can be used clinically in the differential diagnosis of acute abdomen.

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