D-Dimer Levels During Progressive Intestinal Ischaemia: An Experimental Study in Rats

Progresif İntestinal İskemide D-Dimer Düzeyleri: Ratlarda Deneysel Bir Çalışma

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ABSTRACT Objective: Acute mesenteric artery occlusion remains a serious problem in surgical clinics. D-dimer may be a potential marker for the early diagnosis of this disease. We evaluated the association between progressive intestinal ischaemia and plasma D-dimer levels in a rat model. **Material and Methods:** Male Wistar albino rats (n= 40) were randomised into the following groups (n = 10/group): sham-operated group (A); 1-hour occlusion of the anterior mesenteric artery group (B); 3-hour occlusion group (C); and 6-hour occlusion group (D). Intra-cardiac blood samples (1.8 mL each) were obtained for the D-dimer test, and the small intestines were resected for histopathological evaluation at the end of the experiment. **Results:** The D-dimer levels of the four groups were 63.53 ± 10.90, 58.49 ± 8.88, 60.84 ± 11.90, and 63.26 ± 9.74 μg/L respectively. No statistically significant difference in plasma D-dimer levels was observed among groups. However, histopathological examination revealed ischaemia in group B, partial necrosis in group C, and total necrosis in group D. **Conclusion:** No association was found between progressive intestinal ischaemia and plasma D-dimer levels in this rat model.

Key Words: Abdomen, acute; intestinal diseases

ÖZET Amaç: Akut mezenter arter oklüzyonu cerrahi kliniklerde ciddi bir hastalık olarak önemini sürdürmektedir. Bu hastalığın erken tanısında D-dimer potansiyel bir belirteç olabilir. Bu amaçla progresif intestinal iskemi oluşturulmuş ratlarda D-Dimer düzeyleri ile progresif intestinal iskemi arasındaki ilişkiyi araştırdık. **Gereç ve Yöntemler:** Çalışmada her grup için 10 denek olmak üzere toplam 40 adet erkek Wistar albino rat kontrol grubu (A); 1-saatlik mezenter arter oklüzyonu (B); 3-saatlik mezenter arter oklüzyonu (C); ve 6-saatlik mezenter arter oklüzyonu (D) olarak randomize edildi. D-dimer değerlendirmesi için intrakardiyak kan örnekleri alındı (1.8 mL) ve ince bağırsaklar rezeke edilerek histopatolojik değerlendirme yapıldı. **Bulgular:** Gruplardaki D-dimer düzeyleri sırası ile 63.53 ± 10.90; 58.49 ± 8.88; 60.84 ± 11.90 ve 63.26 ± 9.74 μg/L idi. Gruplar arasında istatistiki bir fark yoktu. Histopatolojik olarak Grup B'de iskemik değişiklikler, C'de kısmi nekroz, D'de total nekroz tespit edildi. **Sonuç:** Bu rat modelinde progresif intestinal iskemi ve plazma D-Dimer düzeyleri arasında herhangi bir ilişki bulunamadı.

Anahtar Kelimeler: Karın, akut; intestinal hastalıklar

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Acute mesenteric arterial occlusion remains a serious problem, with high mortality and morbidity, despite advances in diagnosis and therapy over the past decade.[1-3] Prompt recognition, diagnosis, and treatment are crucial for patient survival, and time is the most important factor in preventing necrosis of the ischaemic intestine.

There are no non-invasive diagnostic tools, rapid or easy radiological techniques, or laboratory tests to diagnose acute occlusion of the superior mesenteric artery (SMA). Additionally, serious difficulty may be encountered in detecting ischaemic necrosis in the part of intestinal loop that is newly inserted into the abdomen because of a strangulated hernia.

D-dimer is a fibrin degradation product present in the blood after a blood clot is degraded by fibrinolysis. Thus, its concentration in the blood is related to thrombosis and thrombolysis.[4] Recently, clinical and experimental studies have suggested D-dimer as a potential marker for acute SMA occlusion, acute bowel ischaemia, and necrosis.[5-7]

The aims of this study were to assess D-dimer levels in the blood and pathological changes in intestinal tissue during progressive intestinal ischaemia in a rat model.

MATERIAL AND METHODS

The study was approved by Ondokuz Mayis University, Experimental Research Centre Board for the care of animals. Male adult Wistar albino rats (n = 40, aged 8-9 months) weighing 280-300 g were used. All animals were fed a standard laboratory diet and kept in cages at room temperature (23 ± 1°C) until the day of experiment. During the last 12 h before the experiments, the animals were allowed *ad libitum* access to water, but no solid food. In the morning, rats were randomised into four groups of 10 rats each.

SURGICAL PREPARATION

The rats were anaesthetised by intramuscular injection of 40 mg/kg ketamine (Ketalar flk; Parke-Davis, Detroit, MI). Anaesthesia was maintained by additional doses of ketamine, as required. Each rat was placed in the supine position on a warming pad, and the abdomen was shaved. A temperature probe was placed in the rectum. Rectal temperature was maintained at 37 ± 0.5°C throughout the experiment. All surgical procedures were performed under sterile conditions.

Group A. For the sham-operated group, only a midline laparotomy was performed. After exploration of the abdominal cavity, the abdominal wall was sutured. An intra-cardiac blood sample (1.8 mL each) was obtained for the D-dimer test by puncturing with a 22-gauge injector in the left thoracic region. **Group B.** After a midline laparotomy, the anterior mesenteric artery was prepared and ligated at the origin of the abdominal aorta. The abdominal wall was closed. After 1 hour of occlusion, an intra-cardiac blood sample was obtained. **Group C.** The same procedure was followed as described for group B, except that occlusion was maintained for 3 hours. **Group D.** The same procedure was followed as described for group B, except that occlusion was maintained for 6 hours. All rats were sacrificed by injection of a high dose of Pentothal at the end of the study. The entire small intestine was resected for histopathological evaluation.

Each blood sample (1.8 mL) was collected in a tube that contained 0.7 mL of sodium citrate buffer (Vacutainer; Becton Dickinson, Meylan Cedex, France) to prevent clotting. The blood samples were centrifuged at 2000 rpm for 20 minutes. The D-dimer levels were measured with the latex-enhanced turbidimetric test using a D-dimer Plus Kit (Dade Behring, Marburg, Germany), as used in previous studies.[6,8] The plasma was analysed with a BCS (Dade Behring) analyzer.

Tissue samples from the small intestine were fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Sections were cut at a thickness of 4-6 mm and stained with haematoxylin and eosin.

STATISTICAL ANALYSIS

The Kruskal-Wallis non-parametric variant test was used. P values < 0.05 were deemed to be statistically significant.

RESULTS

The experimental study was carried out ten animals for each group. The D-dimer levels in groups A, B, C, and D were 63.53 ± 10.90, 58.49 ± 8.88, 60.84 ± 11.90, and 63.26 ± 9.74 μg/L, respectively. There was no statistical difference among groups (Table 1; $p > 0.05$).

TABLE 1: D-dimer levels and pathological findings in the rat groups.

	Groups			
	A	**B**	**C**	**D**
	(Sham-operated)	**(1-hour occlusion)**	**(3-hour occlusion)**	**(6-hour occlusion)**
Plasma D-dimer (µg/L)	63.53 ± 10.90	58.49 ± 8.88	60.84 ± 11.90	63.26 ± 9.74
Histopathology	Normal histological features	Atrophy of villi; exfoliation of epithelial lamina; necrosis and focal bleeding at the end of the villus	Complete necrosis of the villi and in the sections leading to the tunica muscularis	Total necrosis of tissues, including the tunica muscularis

Values are mean ± SD. P > 0.05 between the groups.

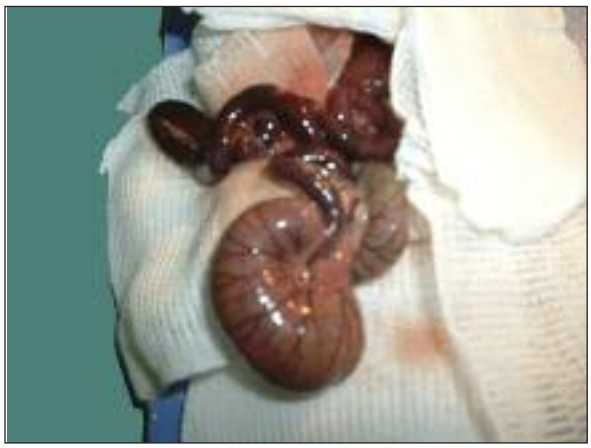

FIGURE 1: A macroscopic view of the small intestine 3 hour after occlusion.

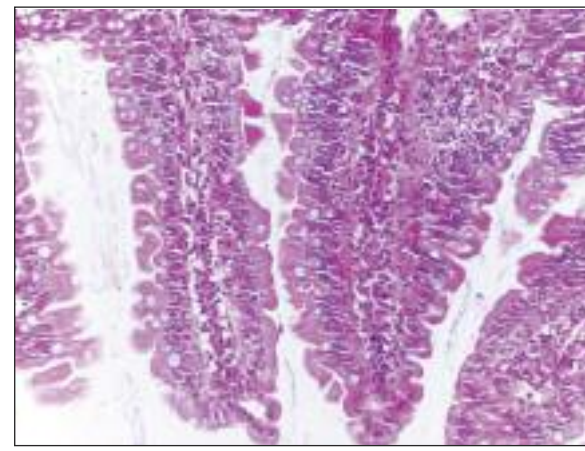

FIGURE 2: The sham-operated group shows normal histological features; haematoxylin and eosin staining, original magnification ×140.

HISTOPATHOLOGICAL EXAMINATION

The small intestine samples were evaluated by a pathologist via light microscopy. Ischaemic changes were observed in group B. Reddish-brown colours and fragile structures were observed in group C (Figure 1). Brown colours and fragile structures were observed in group D.

In group B, atrophy of the villi, exfoliation of the epithelial lamina, and necrosis and focal bleeding at the villus end were evident. In group C, there was complete necrosis of the villi, and the necrosis extended to the tunica muscularis when compared to sham group (Figure 2 and Figure 3). In group D, there was total necrosis of the tunica muscularis mucosa.

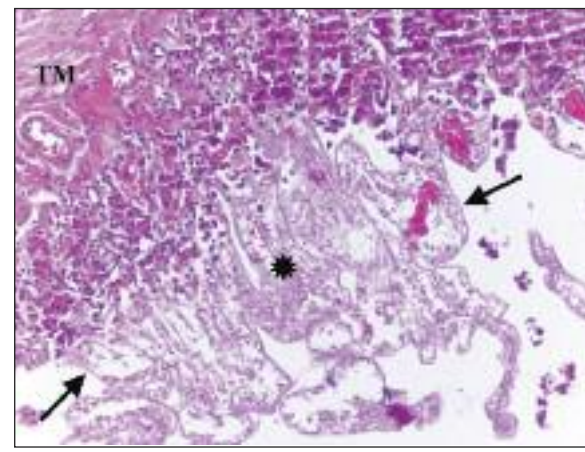

FIGURE 3: Three hours after occlusion, there is complete necrosis of the villi (arrows and star) and the necrosis extends to the tunica muscularis (TM); haematoxylin and eosin staining, original magnification ×140.

DISCUSSION

Acute mesenteric ischaemia is responsible for approximately 0.1% of all hospital admissions. Treatment of patients with intestinal ischaemia as a result of acute mesenteric arterial occlusion, strangulated hernia, or other causes is an important matter

and depends on prompt diagnosis and appropriate patient management.[9,10]

D-dimer is a fibrin degradation product that has been used extensively in the diagnosis of venous thromboembolism. A high level of D-dimer has high sensitivity and negative predictive value for proximal deep vein thrombosis and pulmonary embolism.[5,11,12] However, D-dimer may be produced in a variety of diseases and conditions associated with thrombosis and thrombolysis.[13] It has also been studied as a potential marker for the early diagnosis of acute mesenteric artery occlusion, intestinal ischaemia, and necrosis. Acosta *et al.*[5] reported a preliminary clinical study of acute mesenteric ischaemia, in which six patients with embolic or thrombotic mesenteric disease had significantly higher D-dimer levels than eight patients with different intra-abdominal pathologies. The authors proposed D-dimer as a marker for acute bowel ischaemia. It is hoped that this report raised awareness regarding the importance of the early diagnosis of this potentially fatal disease.

Ischaemia triggers the activation of intravascular fibrinolytic systems, causing a continuous increase in the level of D-dimer as long as this activity continues.[6] Altinyollar et al[6] found high D-dimer levels in rats after ligation of the mesenteric artery. Similarly, Zeybek et al[7] indicated that when the duration of intestinal ischemia was prolonged, serum D-dimer levels increased comparing with the control group, with the difference being statistically significant at 2 hours. These findings suggest that measuring plasma D-dimer levels may be a useful laboratory test for the early diagnosis of acute mesenteric obstruction. In contrast, Kulacoglu et al.[8] reported that the D-dimer level was not adequately supported as an independent parameter for the diagnosis of mesenteric ischaemia. In our study, there was no statistical difference in D-dimer levels among groups. Pathologically, ischaemic changes in the small intestine were observed after 1 hour occlusion, partial necrosis after 3 hours occlusion, and total necrosis in all layers of the small intestine after 6 hours occlusion of the anterior mesenteric artery (Figs. 1, 2, and 3 respectively). Importantly, histopathological findings implicated that the critical time for intestinal viability was 2 and 4 hours post-operatively according to Zeybek et al.[7] It can be thought that intestinal damage could be appeared at 1-3 hours after mesenteric arterial ligation in accordance with our pathological findings.

D-dimer is an acute phase reactant and may be elevated in *all* stressed rats. Any handling, surgery, trauma or even intramuscular injection may cause increased D-dimer levels.[14] In our study, increased D-dimer levels were observed in the laparotomy group, while the mean levels of D-dimer remained roughly the same among study groups (Table 1). At the same time, its levels could lightly be elevated in the group C and D but this increase was not relative to the degree of necrosis observed in the intestinal layers. This may be the result of fibrinolysis-independent mechanisms, such as the previously reported cellular endocytotic uptake of fibrin monomers.[15]

Zeybek et al.[7] investigated the time dependency of the relationship between plasma D-dimer levels and the degree of intestinal necrosis. In a rat model of strangulated hernia, the authors observed elevated D-dimer levels only in the 2-hour occlusion group. They reported serious necrosis of the intestine, but not elevated D-dimer levels, in the 4-, 6-, and 8-hour strangulation groups.

Although prior studies have documented that markers of fibrinolysis are abnormal in people and experimental animals with SMA occlusion, it remains unclear whether these abnormalities reflect fibrinolytic dysfunction. The dynamics of the activation of fibrinolytic and coagulation systems may differ between humans and animal models, including the D-dimer analysis methodology in rats. For example, D-dimer levels were unchanged after constriction of the SMA in a pig model.[14]

In conclusion, no association was found between progressive intestinal ischaemia and plasma D-dimer levels in this rat model. Further clinical studies are necessary to determine the diagnostic test performance of D-dimer such as sensitivity, specificity, reference range, cut-off and predictive value in patients with acute intestinal ischemia.

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