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Diagnostic and prognostic value of procalcitonin and phosphorus in acute mesenteric ischemia

Akut mezenter iskemide prokalsitonin ve fosforun tanısal ve prognostik değeri

Keziban KARABULUT,[1] Mehmet GÜL,[1] Zerrin Defne DÜNDAR,[1]
Başar CANDER,[1] Sevil KURBAN,[2] Hatice TOY[3]

BACKGROUND
In this study, using an animal model of acute mesenteric ischemia (AMI), we investigated the possible use of procalcitonin and phosphorus in the early diagnosis of AMI.

METHODS
In this study, 21 New Zealand rabbits were used. Subjects were allocated into three groups as Control, Sham and Ischemia. No intervention was performed in the subjects in the Control group. In the subjects in the Sham and Ischemia groups, laparotomy was performed with midline incision. In the Ischemia group, the superior mesenteric artery was found and tied after laparotomy. Blood was drawn from the animals in all groups at 0, 1, 3 and 6 hours, and procalcitonin and phosphorus levels were studied in these samples.

RESULTS
In the Ischemia group, the increase in the levels of serum phosphorus and procalcitonin was found to be statistically significant compared to the Control and Sham groups ($p<0.05$). The levels of phosphorus and procalcitonin were detected to increase from the 1st hour after ischemia onset, and the increase continued for the following 6 hours ($p<0.05$).

CONCLUSION
Phosphorus and procalcitonin may be important parameters for use in the early diagnosis and prognosis of AMI.

Key Words: Acute mesenteric ischemia; phosphorus; procalcitonin.

AMAÇ
Akut mezenter iskemi (AMİ) modeli kullanılarak yapılan bu çalışmada, serum prokalsitonin ve fosfor düzeylerinin AMİ erken tanısında kullanılabilirliği araştırıldı.

GEREÇ VE YÖNTEM
Çalışmada 21 adet Yeni Zelanda tavşanı kullanıldı. Denekler Kontrol, Sham ve İskemi grubu olarak adlandırıldı. Kontrol grubundaki deneklere herhangi bir girişim yapılmadı. Sham ve İskemi grubundaki deneklere orta hat insizyonu ile laparotomi yapıldı. İskemi grubundaki deneklere ise laparatomi yapıldıktan sonra süperior mezenterik arter bulunarak bağlandı. Her üç gruptaki hayvanlardan 0., 1., 3. ve 6. saatlerde kan alındı, bu numunelerden prokalsitonin ve fosfor çalışıldı.

BULGULAR
İskemi grubunda, serum fosfor ve prokalsitonin düzeylerindeki yükselme kontrol ve sham gruplarına göre istatistiksel olarak anlamlı bulundu ($p<0,05$). Fosfor ve prokalsitonin düzeylerinin, iskemi oluşturulduktan sonra 1. saatten itibaren arttığı ve bu yüksekliğin 6 saat boyunca devam ettiği saptandı ($p<0,05$).

SONUÇ
Fosfor ve prokalsitonin'in AMİ'nin erken tanısında ve prognozunda kullanılabilecek önemli parametreler olabileceğini düşünüyoruz.

Anahtar Sözcükler: Akut mezenter iskemi; fosfor; prokalsitonin.

Departments of [1]Emergency Medicine, [2]Biochemistry, [3]Pathology, Selcuk University Meram Faculty of Medicine, Konya, Turkey.

Selçuk Üniversitesi Meram Tıp Fakültesi, [1]Acil Tıp Anabilim Dalı, [2]Biyokimya Anabilim Dalı, [3]Patoloji Anabilim Dalı, Konya.

Correspondence *(İletişim):* Keziban Karabulut, M.D. Selçuk Üniversitesi Meram Tıp Fakültesi Acil Tıp Anabilim Dalı, 42100 Konya, Turkey.
Tel: +90 - 332 - 223 72 03 e-mail *(e-posta):* dr_kezi@hotmail.com

Acute mesenteric ischemia (AMI) remains a highly fatal disease (70%) despite the improvements in diagnostic and therapeutic methods.[1] The most important factor affecting the outcome of AMI is the duration of the ischemia. Diagnosis must be made immediately in case of suspicion in these patients. Re-instating the blood supply of the bowel in the first 6 hours (h) of ischemia improves the prognosis, especially in emboli-related ischemia. Bacterial and endotoxin absorption causing an inflammatory response increases as the duration of ischemia prolongs. Ischemia progresses and results in sepsis, acidosis, septic shock, and finally death.[2]

In AMI, in which early diagnosis is essential, the common result of studies on biochemical markers carried out in recent years is that there is a lack of a sensitive and specific marker with diagnostic potential, sufficient to increase survival. The optimum biochemical marker for the early diagnosis of AMI must be released from the intestinal mucosa, must avoid the hepatic first-pass effect, and must be detected in the peripheral blood. Novel diagnostic markers studied in recent years based on this opinion are promising.[3-5] Procalcitonin is a protein with a molecular weight of 13 kDa, consisting of 116 amino acids. Its levels increase in severe bacterial, fungal and parasitic infections, autoimmune diseases, sepsis, and multi-organ deficiency syndrome (MODS). Procalcitonin is a pro-inflammatory cytokine-like mediator. Its expression is regulated by pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6.[6] One of the most promising parameters in the early diagnosis of AMI is the serum phosphorus level, which increases just after the occlusion of the mesenteric artery due to release of intracellular phosphorus into the circulation in ischemic injury.[7]

In this experimental study, we investigated the possible roles of two markers (procalcitonin and phosphorus) in the early diagnosis and prognosis of AMI by determining the short-term alterations in their levels after development of ischemia.

MATERIALS AND METHODS

Approval was obtained from the Experimental Animals Ethics Committee of the Experimental Medicine Research and Training Center. The study was carried out in Selcuk University Experimental Medicine Research and Training Center.

A total of 21 New Zealand rabbits weighing 3000-3500 g were used in the study. The animals were fed with the same standard feed until 12 h before the experiment. They were fasted for 12 h before beginning the test. The subjects were randomly divided into 3 groups of 7 animals each as Control, Sham and Ischemia groups.

Ketamine (50 mg/kg) and xylazine (15 mg/kg) were administered into the hind legs of the subjects in all 3 groups. Vascular access was established on the dorsal auricular veins of the animals using a 22 G needle after anesthesia was provided with the aim of drawing blood and administering fluid. Without any interventions, 3 ml of blood was drawn into gel Vacutainer tubes in order to study procalcitonin and phosphorus at 0, 1, 3, and 6 h in the subjects in the Control group. 5 ml of 0.9% normal saline was administered using the same vascular access following every blood draw. Tissue samples were not obtained from this group.

Blood was similarly drawn at 0 h from the subjects of the Sham and Ischemia groups. The abdominal sites of the subjects in each of the two groups were shaved and cleaned using 10% povidone iodine. Laparotomy was performed through a midline incision. The peritoneum was passed in the Sham group. Thereafter, the abdominal wall and the peritoneum were sutured using 2/0 silk. In the Ischemia group, the superior mesenteric artery (SMA) was found and ligated using 0 silk following laparotomy. Thereafter, the abdominal wall and the peritoneum were closed by suturing. 3 ml of blood was drawn from the subjects of the two groups at 0, 1, 3, and 6 h following these procedures. 5 ml of 0.9% normal saline was administered following every blood draw. Tissue samples were not obtained from the Sham group. Subjects in the Ischemia group were sacrificed by administering 50 ml/kg of ketamine intravenously (IV). After the subjects were sacrificed, 10-cm distal ileum specimens were placed in 10% formaldehyde solution after having been washed with normal saline solution for histopathological examination. Tissue specimens were stained with hematoxylin-eosin as paraffin blocks and examined under light microscopy.

Sample Preparation

Every 5 ml of blood sample placed into gel Vacutainer tubes was centrifuged at 3000 rpm for 10 minutes (min), and then waited for 30 min for coagulation. The obtained serum samples were placed in Eppendorf tubes by pipetting. 10 cm distal ileum specimens obtained for histopathological examination were fixed with 10% formaldehyde solution after having been washed with normal saline solution and embedded in paraffin blocks following routine xylol-alcohol series.

Evaluation of Samples

Biochemical Evaluation

An ELISA kit appropriate for determination of procalcitonin (ELISA kit for procalcitonin E0689 Uscn Life Science Inc., Wuhan) was used. A routine biochemistry kit was used for determination of serum phosphorus levels.

Histopathological Evaluation

On microscopic evaluation of the subjects, it was found that the pulse in the SMA disappeared immediately after ligation and the bowel turned pale. Tissue samples obtained from the Ischemia group at the end of the 6th hour for histopathological examination were evaluated under light microscopy with 100x magnification after staining with hematoxylin-eosin. Mucosal injury was graded according to the scoring system determined by Chiu et al.[8]

Statistical Analysis

The collected data were recorded in previously prepared forms. Statistical analyses were performed using the SPSS 16.0 package program. Inter-group comparisons were made using the variance analysis (ANOVA) post-hoc Tukey test in repeated measurements. The Bonferroni correction paired t test was used to determine the difference between measurements. A p value of <0.05 was considered statistically significant.

RESULTS

Serum Procalcitonin Values

Serum procalcitonin values at 0, 1, 3, and 6 h were significantly higher in the Ischemia group compared to the Control and Sham groups (p=0.003 for both) (Table 1). No statistically significant difference was found between the Control and Sham groups (p=0.809). In the Ischemia group, serum procalcitonin values were found to be higher at 1, 3 and 6 h compared to 0 h, and these increases were found to be statistically significant (p=0.008 at 1 h, p=0.01 at 3 h, p=0.02 at 6 h) (Fig. 1).

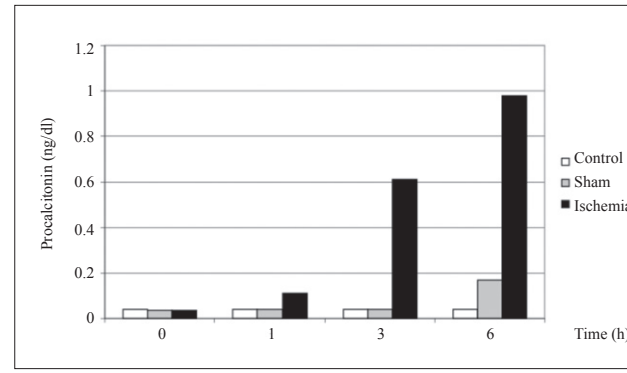

Fig. 1. Time-dependent changes of serum procalcitonin levels.

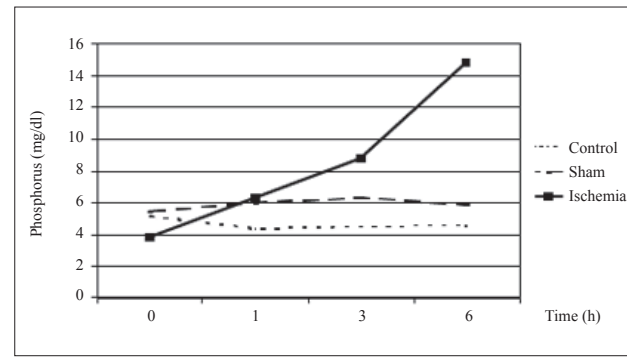

Fig. 2. Time-dependent changes in serum phosphorus levels.

Serum Phosphorus Values

Serum phosphorus values at 0, 1, 3, and 6 h were significantly higher in the Ischemia group compared to the Control and Sham groups (p=0.001 for both) (Table 2). A statistically significant difference was found between the Control and Sham groups (p>0.05). Increases in serum phosphorus levels at 1, 3 and 6 h were found to be statistically significant compared to 0 h levels (p=0.002 at 1 h, p=0.00 at 3 h, p=0.00 at 6 h) (Fig. 2).

Histopathological Findings

On histopathological examination of bowel tissues of 7 rabbits from the Ischemia group, bowel tissues of 5 rabbits (71.4%) were evaluated as Grade 5 (hemorrhage, ulceration and necrosis in the lamina propria)

Table 1. Mean, standard error, %95 interval values of procalcitonin in groups

Groups	Time (h)	Mean (ng/dl)	Std. error	%95 interval
Control	0	0.03	0.001	0.03-0.04
	1	0.03	0.009	0.03-0.04
	3	0.04	0.001	0.03-0.04
	6	0.04	0.001	0.03-0.04
Sham	0	0.03	0.001	0.03-0.04
	1	0.04	0.001	0.04-0.05
	3	0.04	0.002	0.04-0.05
	6	0.17	0.11	0.11-0.45
Ischemia*	0	0.03	0.005	0.04-0.06
	1	0.11	0.01	0.06-0.15
	3	0.61	0.17	0.19-1.03
	6	0.98	0.17	0.4-1.3

*p values determined for the comparison of time-dependent procalcitonin levels in the ischemia group; p=0.008 for hour 1, p=0.01 for hour 3, p=0.02 for hour 6.

Table 2. The serum phosphorus levels (mg/dl)

Phosphorus	0. hour	1. hour	3. hour	6. hour
Control	5.14±0.58	4.32±0.39	4.45±0.61	4.55±0.571
Sham	5.42±1.16	5.94±0.52	6.25±0.76	5.81±0.69
Ischemia*	3.8±0.67	6.25±1.67	8.81±0.70	14.78±1.77

*p values determined for the comparison of time-dependent procalcitonin levels in the ischemia group; p=0.002 for hour 1, p=0.00 for hour 3, p=0.00 for hour 6.

(Fig. 3); bowel tissues of 2 rabbits (28.6%) were evaluated as Grade 4 (ulceration in villus) (Fig. 4).

DISCUSSION

Acute mesenteric ischemia (AMI) is a clinical condition that must be diagnosed immediately due to the high mortality rate. Diagnosis is the most important step in the course of the disease due to the insignificant and non-specific clinical findings and limited diagnostic tests.[9] Systemic inflammatory response syndrome and septic complications are usually responsible for the high mortality in AMI.[10] Studies aimed at finding a specific biochemical, serological parameter in the early diagnosis of AMI have been intensified recently.[11]

Many laboratory parameters used in the diagnosis of inflammatory diseases indicating the immune response are available. Procalcitonin is a novel parameter that has been added to the infection markers in recent years. Procalcitonin is encountered as an early increasing marker in sepsis and serious infections compared to the inflammatory response parameters such as body temperature, C-reactive protein (CRP) and white blood cell count.[12] With regard to AMI, many studies are available on ILs, TNF-α and CRP of pro-inflammatory cytokines. The increase in blood cytokine levels in AMI indicates that the systemic response appears in the early stages of ischemia, and rather than making the diagnosis, it is valuable in terms of prognosis of the patients.[13,14] In one study, serum IL-6 levels were found to be higher in patients with the diagnosis of AMI compared to the healthy control group.[15] In another study performed by creating SMA occlusion in rats, a significant increase was found in TNF-α, IL-6 and IL-1 levels beginning from the 2nd hour in the ischemia group compared to control and laparotomy groups.[16]

The procalcitonin level is below detectable values (<0.1 ng/ml); all values above 0.5 ng/ml are considered pathological. Procalcitonin production can be stimulated by bacterial endotoxins, exotoxins and some cytokines. Procalcitonin has been shown to increase before CRP and after TNF-α and IL-6 in acute inflammatory conditions.[12]

In previous studies, injection of a small amount of bacterial endotoxin was found to stimulate procalcitonin production in healthy individuals. Procalcitonin levels reach detectable values after 2-3 hours, increase rapidly in 6-8 hours and reach their peak value in 12 hours. The levels remain the same for approximately 12 hours. Thereafter, the levels decrease to the normal level in two days. Half-life of procalcitonin varies between 20-24 hours.[17]

In a study comparing procalcitonin levels with CRP, TNF-α and IL-6 levels, procalcitonin was detected to increase before CRP. When this condition was adapted to clinics, procalcitonin was considered to be a better indicator for detection of early stage infections compared to CRP. On the other hand, cytokines like TNF-α and IL-6 increase in the early stage. However, procalcitonin is superior to these cytokines for determination of infections owing to their significantly shorter half-lives.[18]

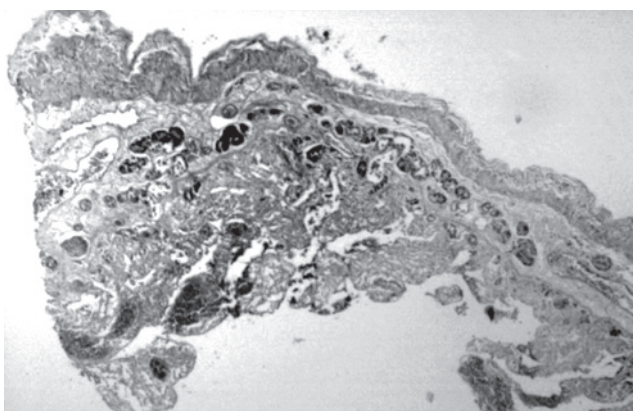

Fig. 3. Grade 5 hemorrhage, ulceration and necrosis in the lamina propria (H-E x 100).

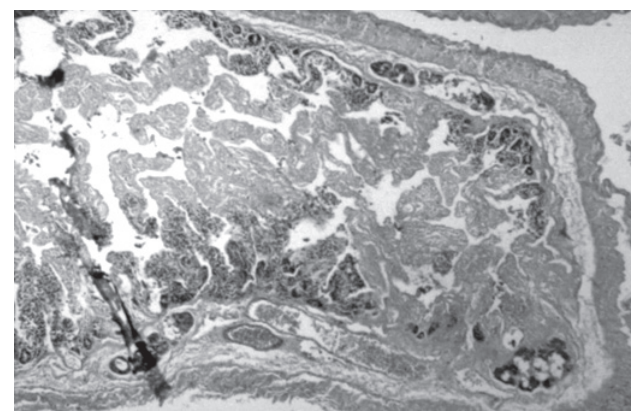

Fig. 4. Grade 4 uulceration in villus (H-E x 100).

In a study performed by creating bowel strangulation in rabbits, procalcitonin levels were analyzed and found to be higher in the group with strangulation compared to the normal group. An increase was detected in procalcitonin levels at the 30th and 60th minutes of the study, and it was found to peak at the 120th minute.[19] In another study, procalcitonin levels were detected to be higher in inflammatory bowel disease - Crohn's disease compared to normal individuals.[20]

Studies and information about the course of procalcitonin in AMI and acute ischemic conditions are limited. There is no experimental study in the literature in which procalcitonin levels were assessed in AMI. A few published studies are available on procalcitonin in acute myocardial infarction and acute stroke. In the study of Kafkas et al.,[21] procalcitonin levels were found to be high in the early stage of acute myocardial infarction. In another study, serum procalcitonin levels were evaluated in patients experiencing acute stroke, and an increase in serum procalcitonin levels was detected beginning from the 1st day, with the peak level reached on the 7th day.[22]

In this study, procalcitonin levels were evaluated in AMI, which is an ischemic and acute inflammatory disease of the bowel. Carrying out the study on rabbits enabled repetitive blood to be drawn from the same subject, and thus the effect of ischemia duration on procalcitonin levels could be evaluated more accurately. In the study, a minimal increase was detected in the procalcitonin levels at 1 hour. This increase continued over the following hours and reached more significant levels.

In AMI, it was detected in clinical and experimental studies that phosphorus diffused into the circulation as a result of ischemic injury of intestinal tissue, renal phosphorus excretion decreased following ischemia, and the hepatic clearance of phosphorus decreased related to decreased effective perfusion.[7] While the serum phosphorus levels were reported to be high as early as 1 hour after the onset of mesenteric ischemia in some previous studies,[7] other studies reported that the phosphorus level increased only at 3-4 hours following ischemia. In the experimental study of Lores et al.[23] performed on dogs, they found that an increase in the phosphorus levels could be an indicator of an early diagnosis of ischemia; however, the phosphorus levels significantly increased at the 4th hour of ischemia. In another study performed on 28 rabbits, inorganic phosphorus levels were found to increase 2 hours following ischemia, and this increase continued for 24 hours.[24]

In a meta-analysis of 20 studies reviewing 18 different biochemical markers, the specificity and sensitivity of serum phosphorus levels in the diagnosis of AMI were found as 82% and 26%, respectively.[3]

In this study, a significant increase in serum phosphorus levels was detected beginning from the 3rd hour in the Ischemia group, consistent with the literature. A significant increase was detected at 6 hours compared to 0, 1 and 3 hours. When compared to the Control and the Sham groups, serum phosphorus was found to increase significantly in the Ischemia group.

In this study, procalcitonin levels were observed to begin increasing as of the 1st hour in the AMI model, and this increase was found to continue in the following hours. Furthermore, a significant increase was found in the phosphorus levels beginning from the 3rd hour.

In conclusion, procalcitonin and phosphorus, which have a prognostic value in inflammatory conditions, can have a significant value in the early diagnosis and prognosis of AMI. However, we believe that further studies are needed in which the other ischemic and inflammatory abdominal pains along with AMI are compared in terms of procalcitonin and phosphorus levels. Supportive clinical and experimental studies on this issue must be carried out.

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