

## Lipoprotein-associated phospholipase-A<sub>2</sub> can be a diagnostic marker in the early stage diagnosis of acute mesenteric ischemia

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**Background/aim:** The purpose of this experimental study was to investigate the role of lipoprotein-associated phospholipase-A<sub>2</sub> (Lp-PLA<sub>2</sub>) in the diagnosis of acute mesenteric ischemia (AMI) in the early stage.

**Materials and methods:** Twenty-seven New Zealand rabbits were randomly divided into 3 groups in this study. Blood specimens were obtained from the groups at hours 0, 1, 3, and 6. Using the blood samples drawn from all groups, Lp-PLA<sub>2</sub> and C-reactive protein (CRP) parameters were investigated.

**Results:** There was a significant rise in the levels of both Lp-PLA<sub>2</sub> and CRP starting at hour 1 ( $P < 0.05$ ) (hour 1; Lp-PLA<sub>2</sub>,  $P = 0.003$ ) in the ischemia group. In the sham group, the levels of Lp-PLA<sub>2</sub> and CRP started to rise at hour 3 ( $P < 0.05$ ) (hour 3; Lp-PLA<sub>2</sub>,  $P = 0.011$ ). At hour 6 of ischemia, the area under the ROC curve was 100%, and the cut-off value of 63.91 ng/mL revealed a sensitivity of 88% and a specificity of 100% for Lp-PLA<sub>2</sub>.

**Conclusion:** These findings showed the role of serum Lp-PLA<sub>2</sub> and CRP levels in the early diagnosis of AMI. Thus, further studies are needed to describe the role of Lp-PLA<sub>2</sub> in the early diagnosis of AMI.

**Key words:** Acute mesenteric ischemia, lipoprotein-associated phospholipase-A<sub>2</sub>, C-reactive protein, experimental study

### 1. Introduction

Acute mesenteric ischemia (AMI), which is associated with a high mortality rate, should be considered as a causative clinical condition because of its difficult diagnosis (1–3). The symptoms and physical examination findings of AMI are usually nonspecific and radiological changes in the early stage are nondiagnostic. AMI comprises 1%–2% of all gastrointestinal disorders and has a very high mortality rate (71%). Research shows that AMI has severe complications and may be fatal (2,3).

AMI is a life-threatening disorder so that early diagnosis of AMI is very important. However, there are no sensitive and specific biomarkers for use in early diagnosis.

Research on atherosclerosis shows that inflammation has a significant role in the disease and many studies have focused on it. Lipoprotein-associated phospholipase-A<sub>2</sub> (Lp-PLA<sub>2</sub>), which is a current biomarker of inflammation and atherosclerosis, is important for diagnosis and its importance is growing. Lp-PLA<sub>2</sub> is a member of the phospholipase A<sub>2</sub> family. This enzyme is released by inflammatory myeloid cells that rapidly accumulate in the lesion and are associated with atherogenic lipoproteins in

circulation (4). Lp-PLA<sub>2</sub> hydrolyzes the proinflammatory oxidized phospholipids that cause endothelial dysfunction, plaque inflammation, and formation of necrotic focus within the plaque. Furthermore, Lp-PLA<sub>2</sub> is thought to be responsible for the association between an increased inflammatory response in the intima and oxidative change of low-density lipoproteins (LDLs) (5). There are many studies about the effects of Lp-PLA<sub>2</sub> on atherosclerosis. However, as far as we know, there is no study in the literature on the association of Lp-PLA<sub>2</sub> with AMI, which is an atherosclerotic disorder.

The purpose of this experimental study was to determine whether Lp-PLA<sub>2</sub> is of diagnostic value for AMI. Previous studies revealed that Lp-PLA<sub>2</sub> is a novel biomarker for inflammation. The results of Lp-PLA<sub>2</sub> were compared with those of C-reactive protein (CRP), which is a nonspecific inflammatory marker and increases in AMI.

### 2. Materials and methods

This experimental study was approved by the Ethics Committee for Experimental Animals, Center for Research and Application in Experimental Medicine of Necmettin

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Erbakan University, on 20 January 2012, being registered with number 2012-011. The study was performed at the Center for Research and Application in Experimental Medicine of Necmettin Erbakan University in May 2012. All animals were kept and fed by specialized animal care workers under physical conditions recommended by international guidelines.

We applied to the University Ethics Committee to use 10 rabbits in each group in the study. However, the ethics committee allowed 7 rabbits for the control group and 10 rabbits for each of the other groups. The rabbits were randomly divided into three groups. However, because a rabbit died in the ischemia group in the very beginning of the study, that rabbit was excluded from the study. The study carried on with 7 rabbits in the control group, 10 in the sham group, and 9 in the ischemia group, adding up to a total of 26 adult New Zealand rabbits weighing 2500–3000 g each.

All groups received 50 mg/kg ketamine and 15 mg/kg xylazine i.m. in the hind leg. Following anesthesia, vascular access was established in the dorsal ear vein with an Intracath 22G for blood drawing and liquid injections. For the biochemical tests, each time, 5 mL of blood was drawn into gel-containing vacutainer tubes at hours 0, 1, 3, and 6. Following each blood draw, 5 mL of saline was injected into the rabbit through the same vein.

No tissue specimens were obtained from the rabbits in the control group (group I).

In the sham group (group II), after the first blood specimen was obtained, the abdominal wall of each rabbit was shaved and cleaned with 10% povidone-iodine. An abdominal laparotomy was performed with a midline incision. Following laparotomy, the abdominal wall and the peritoneum were closed with 2/0 silk thread sutures. No tissue specimens were obtained from the rabbits in this group either.

In the ischemia group (group III) the same preparations carried out on the rabbits as in the sham group were performed. After having obtained blood specimens at hour 0, each rabbit underwent laparotomy through a midline incision, and the superior mesenteric artery was ligated with a 0 silk thread. The peritoneum and the abdominal wall were then closed up with 2/0 silk sutures. At the end of hour 6 of ischemia, the rabbits were euthanized with high-dose ketamine, and 10-cm-long specimens were obtained from the distal ileum for histopathological examination. The tissue specimens were washed with sterile saline and kept in 10% formaldehyde solution.

### 2.1. Collection of specimens

First 5 mL of blood was obtained from every rabbit and placed in tubes containing EDTA. The blood specimens were then centrifuged at 3000 rpm at 4 °C for 10 min, and the plasma that was separated with this cold centrifugation

was placed in microcentrifuge tubes (Eppendorf) and stored at –80 °C until the time of use. The 10-cm-long specimens of the distal ileum that had been obtained for histopathological examination, in the line of order, were placed in routine xylol-alcohol solution, embedded in paraffin blocks, washed with saline, and fixed in 10% formaldehyde solution.

### 2.2. Biochemical method

Commercial kits and ELISA were used for biomarker measurements. For Lp-LPA<sub>2</sub> measurements, the scientific research special Eastbiopharm Rabbit Lp-PLA<sub>2</sub> ELISA kit (Lot No: 20121024) was used, and for CRP measurements, the scientific research special Eastbiopharm Rabbit Lp-PLA<sub>2</sub> ELISA kit (Lot No: 20121024) was used.

### 2.3. Histopathological examination and evaluation

At the end of the 6-hour ischemia, blood specimens were obtained from the rabbits, and then the rabbits were euthanized. Intestinal specimens for histopathological examination were obtained from the distal ileum. The intestinal tissue specimens were consistent with ischemia macroscopy. The 10-cm-long specimens from the distal ileum were first washed with saline and then fixed in formaldehyde solution. After passing through routine xylol-alcohol series and paraffin embedding, the tissue specimens were cut into 5- $\mu$ m sections. The sections were stained with hematoxylin–eosin and then studied under 100 $\times$  light microscope magnification to score the intestinal mucosal injury on a scale described by Chiu et al.(6).

Scale of mucosal injury

Grade 0: Normal mucosal villi

Grade 1: Extension of the subepithelial space, capillary congestion at the apex of villi

Grade 2: Subepithelial congestion extending to the base of villi

Grade 3: Ulcerations at the apex of some villi, extensive subepithelial congestion

Grade 4: Villus ulcerations, dilated capillaries in the lamina propria

Grade 5: Digestion and disintegration of the lamina propria, hemorrhage, and ulceration

### 2.4. Statistical analysis

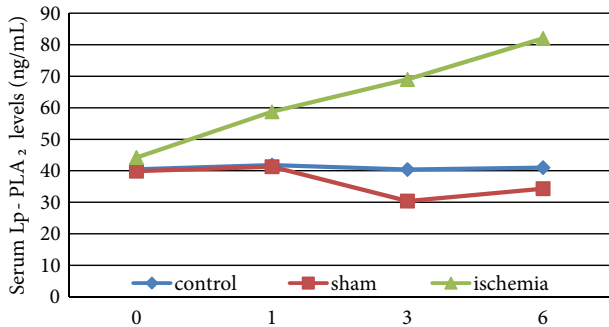
All of the obtained data were recorded on ready tables. SPSS 16.0 was used for the statistical analysis. The Kruskal–Wallis variance analysis and the Mann–Whitney U test were utilized for the comparison of groups. The change in biomarkers over time was evaluated using Friedman's test. Spearman's correlation test was performed for the determination of association between Lp-LPA<sub>2</sub> and CRP levels. Results were considered to be statistically significant at  $P < 0.05$ . The area under the receiver operating characteristic (ROC) curve was used as a performance measure. The results are presented in tables and graphics.

**3. Results**

The serum Lp-PLA<sub>2</sub> and CRP levels were measured in the blood samples at hours 0, 1, 3, and 6 in all rabbits (n = 26). The comparison of the groups in terms of P values of serum Lp-PLA<sub>2</sub> and CRP levels is shown in Table 1, and the change in time in serum Lp-PLA<sub>2</sub> levels is presented in Figure 1.

There were significant differences in the Lp-PLA<sub>2</sub> levels at hours 1, 3, and 6 among the control, sham, and ischemia groups (P < 0.05) (Table 1). It was observed that there were significant differences in the Lp-PLA<sub>2</sub> levels at hours 3 and 6 between the control and sham groups (P < 0.05), and at hours 1, 3, and 6 between the control and the ischemia groups, and between the sham and the ischemia groups (P < 0.05) (Table 1).

There were significant differences in the CPR levels at hours 1, 3, and 6 between the sham and the ischemia groups (P < 0.05) (Table 1), between the control and the ischemia groups, and between the sham and the ischemia groups (P < 0.05) (Table 1). The comparison of the groups in terms of P values of serum Lp-PLA<sub>2</sub> and CRP levels is shown in Table 2 and the change in the CRP level over time is presented in Figure 2.



**Figure 1.** Time-dependent changes of serum Lp-PLA<sub>2</sub> levels.

**Table 1.** Comparison of Lp-PLA<sub>2</sub> and CRP levels between groups.

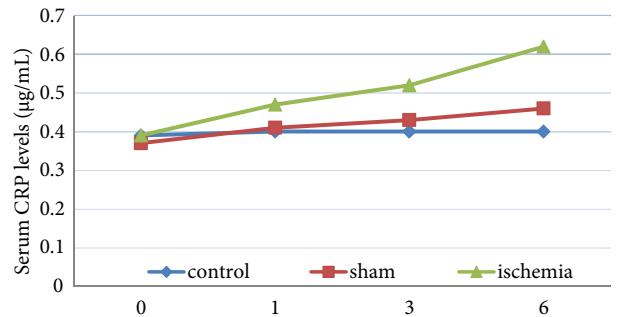
	L0	L1	L3	L6	C0	C1	C3	C6
Kruskal–Wallis Con-Sh-Isch, P	0.742	0.007	0.001	0.001	0.104	0.045	0.001	0.001
Mann–Whitney U								
Con-Sh, P	0.463	1.000	0.003	0.032	0.060	0.920	0.020	0.003
Con-Isch, P	0.711	0.010	0.003	0.001	0.419	0.048	0.001	0.001
Sh-Isch, P	0.462	0.006	0.001	0.001	0.117	0.026	0.001	0.001

L0: Hour 0 Lp-PLA<sub>2</sub>; L1: Hour 1 Lp-PLA<sub>2</sub>; L3: Hour 3 Lp-PLA<sub>2</sub>; L6: Hour 6 Lp-PLA<sub>2</sub>.

C0: Hour 0 CRP; C1: Hour 1 CRP; C3: Hour 3 CRP; C6: Hour 6 CRP. P < 0.05 value is considered significant. Con = Control group; Sh = Sham group; Isch = Ischemia group.

**Table 2.** The changes of markers in time.

Group	Friedman’s test	P
Control	L0-L1-L3-L6	0.478
	C0-C1-C3-C6	0.069
Sham	L0-L1-L3-L6	0.003
	L0-L1	0.527
	L0-L3	0.011
	C0-C1-C3-C6	0.000
Ischemia	L0-L1-L3-L6	0.000
	L0-L1	0.003
	C0-C1-C3-C6	0.000



**Figure 2.** Time-dependent changes of serum CRP levels.

There was no difference between the Lp-PLA<sub>2</sub> and CRP levels at hours 0, 1, 3, or 6 in the control group (P > 0.05). The changes in serum Lp-PLA<sub>2</sub> and CRP levels over time in the groups are shown in Table 3.

In the sham group, there were differences between the Lp-PLA<sub>2</sub> levels at hours 0, 1, 3, and 6 (P < 0.05); there was no difference between the levels at hours 0 and 1, but there

**Table 3.** Mean, median, and standard error values of Lp-PLA<sub>2</sub> and CRP in groups.

Group	Control		Sham		Ischemia	
	Mean	Median	Mean	Median	Mean	Median
L0	40.49 ± 0.26	40.52	39.83 ± 3.2	40.64	44.24 ± 9.08	47.04
L1	41.82 ± 0.57	41.76	41.32 ± 5.66	41.47	58.73 ± 12.86	61.24
L3	40.36 ± 1.88	39.33	30.43 ± 7.89	31.57	68.96 ± 15.60	70.06
L6	41.02 ± 1.44	40.38	34.27 ± 6.27	33.52	82.04 ± 12.21	84.72
C0	0.39 ± 0.02	0.40	0.37 ± .02	0.37	0.39 ± .02	0.39
C1	0.40 ± 0.02	0.41	0.41 ± .02	0.41	0.47 ± .07	0.45
C3	0.40 ± 0.02	0.41	0.43 ± .02	0.43	0.52 ± .05	0.51
C6	0.40 ± 0.01	0.41	0.46 ± .03	0.46	0.62 ± .04	0.62

L0: Hour 0 Lp-PLA<sub>2</sub>; L1: Hour 1 Lp-PLA<sub>2</sub>; L3: Hour 3 Lp-PLA<sub>2</sub>; L6: Hour 6 Lp-PLA<sub>2</sub>; C0: Hour 0 CRP; C1: Hour 1 CRP; C3: Hour 3 CRP; C6: Hour 6 CRP. The unit weight of Lp-PLA<sub>2</sub> is ng/mL; and the unit weight for CRP is µg/mL ± standard deviation.

was a difference between the levels at hours 0 and 3. There were also differences between the CRP levels at hours 0, 1, 3, and 6 ( $P < 0.05$ ) (Table 2).

In the ischemia group, there were differences between the Lp-PLA<sub>2</sub> levels at hours 0, 1, 3, and 6 ( $P < 0.05$ ), starting at hour 1. The differences were observed between the CRP levels at hours 0, 1, 3, and 6 ( $P < 0.05$ ), also starting at hour 1 (Table 2).

A positive correlation was observed between the Lp-PLA<sub>2</sub> and CRP levels ( $r^2 = 0.737$ ,  $P < 0.001$ ) (Figure 3).

The ROC curve was used to measure and compare performance. In the ischemia group, the area under the curve for Lp-PLA<sub>2</sub> at hour 6 was 100%. The cut-off value of 63.91 ng/mL provided a sensitivity of 88% and a specificity of 100% (Figure 4).

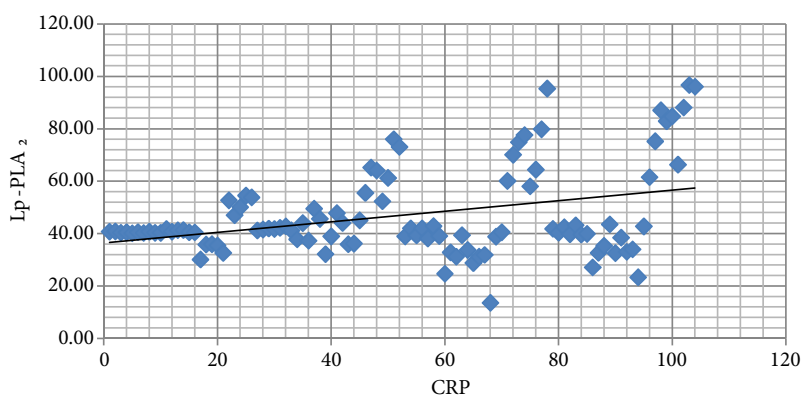
According to the results of histopathological examination of tissue specimens from the distal ileum, the

mucosal injury was of grade 5 in 5 specimens and of grade 4 in 4 specimens (Figure 5).

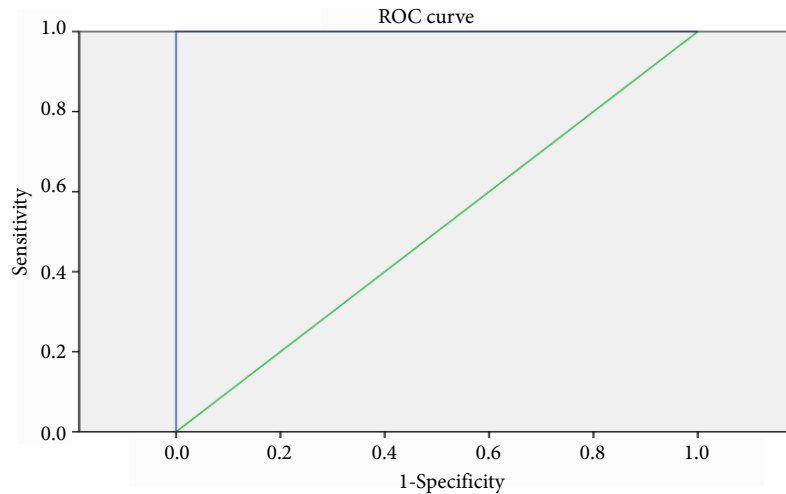
#### 4. Discussion

The prognosis of patients diagnosed with AMI has improved with advances in diagnostics such as imaging techniques and qualified care of intensive care units. Various biomarkers have been used for the diagnosis of AMI, but none of these have been specific for AMI, yielding positive results in local or systemic inflammations. Acosta (7) reviewed the current parameters used in AMI, in both humans and animals, but he did not mention Lp-PLA<sub>2</sub> as a parameter.

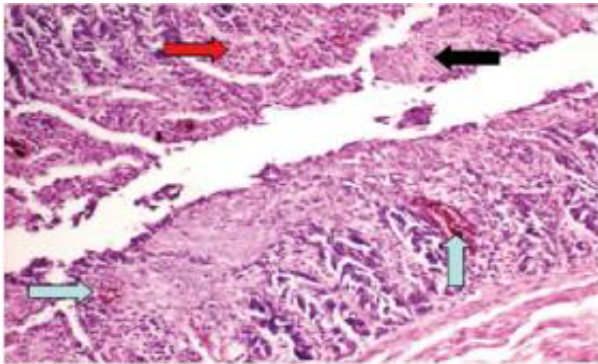
The most important stage of AMI, for certain, is mesenteric ischemia requiring prompt diagnosis before intestinal infarction develops. In view of the high fatality rates, studies on diagnostic tests for AMI have accelerated



**Figure 3.** The relationship between serum Lp-PLA<sub>2</sub> and CRP levels ( $r^2 = 0.737$ ).



**Figure 4.** The ROC curve of Lp-PLA<sub>2</sub> at hour 6 of ischemia.



**Figure 5.** Hemorrhage, ulceration, and necrosis in the lamina propria (H-E ×100).

in recent years. The common result of all these studies is that there is still no sensitive and specific biomarker for clinical use that would indicate the early stage of AMI.

The diagnostic method to be used for AMI in the clinic should be noninvasive, it should not take long to perform, and should rapidly give the results.

Lp-LPA<sub>2</sub> is used and studied in many disorders such as oxidative stress, atherosclerosis, coronary artery disease, cardiac insufficiency, Alzheimer disease, transient ischemic attack (8), cerebrovascular disorder (9), allergic lung diseases, HIV-positive patients, intracranial hemorrhage in preterm infants (10), hypertensive patients, subarachnoid hemorrhage, nonsmall cell lung cancer (11), patients with African dengue (12), hemodialysis patients, diabetes mellitus, multiple sclerosis with immune response (13), polycystic ovary syndrome (14), systemic sclerosis (15), nonalcoholic fatty liver disease, high-grade carotid stenosis with unstable plaque, and metabolic syndrome.

The studies on Lp-PLA<sub>2</sub> have been mainly related to disorders caused by oxidative stress, atherosclerosis, and inflammation. Oxidative stress and inflammation are widely seen in AMI. Lp-PLA<sub>2</sub> is accepted as a biomarker specific for vascular inflammation, and hence it can increase in AMI as well. Even a small elevation in the plasma Lp-LPA<sub>2</sub> level can indicate the presence of plaque inflammation and endothelial dysfunction. The Food and Drug Administration (FDA) has approved the use of Lp-PLA<sub>2</sub> in determining the risk for ischemic attack and coronary artery disease (16).

Dohi et al. (17) studied the association between plaque volume and circulatory Lp-PLA<sub>2</sub> after percutaneous coronary intervention in acute coronary syndrome (ACS) patients and concluded that the levels of circulatory Lp-PLA<sub>2</sub> were associated with changes in plaque volume in patients with ACS.

Lp-PLA<sub>2</sub> has been described as an independent inflammatory risk biomarker for recurrence of ischemic stroke and myocardial infarction (16). Rosso et al. (18) found higher levels of Lp-PLA<sub>2</sub> in intracerebral hemorrhage than in ischemic stroke, and described this finding as a surprise.

In an experimental study, Dündar et al. (19) compared the levels of serum ischemia-modified albumin (IMA) and interleukin-6 (IL-6) in the course of time in cases with AMI. The authors found significantly higher levels of serum IMA and IL-6 in the ischemia group than in the control and the sham groups. Similar to our findings, this study (19) has found inflammatory markers to be significant during the inflammatory stage leading up to AMI.

In our study, Lp-PLA<sub>2</sub> levels were increased at the 1st hour period in the ischemia group but a similar

increase was not observed in the control or sham groups. Furthermore, in the sham group Lp-PLA<sub>2</sub> levels indicated a small decrease at hour 3. This can be explained by the antioxidant system. This system was able to overcome oxidative stress and the inflammatory process, which develop more lightly and slowly in the sham group when compared with the ischemia group. These results demonstrated that increased levels of Lp-PLA<sub>2</sub> in the early stage of AMI can be helpful in diagnosis.

An experimental study on intestinal ischemia-reperfusion found that the phospholipase A2 and Lp-PLA<sub>2</sub> levels were higher in the group resulting in death following

reperfusion than in the sham group (20). The present study suggests that Lp-PLA<sub>2</sub> increases in the presence of inflammatory reaction and oxidative stress.

As a result of this study, increased Lp-PLA<sub>2</sub> levels were obtained in AMI. Furthermore, Lp-PLA<sub>2</sub> levels were correlated with CRP, which is another nonspecific inflammatory biomarker.

The blood Lp-PLA<sub>2</sub> levels were increased in the first hours of AMI. This increase is consistent with the increase in CRP levels. The results of this study demonstrated that Lp-PLA<sub>2</sub> can be used in the early diagnosis of AMI. More comprehensive studies on the subject are needed.

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