






# Research of importance of thiol, CRP, and lactate in diagnosing mesenteric ischemia at an early stage: animal experiment model

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## ABSTRACT

**BACKGROUND:** Acute mesenteric ischemia is especially seen in the elderly population. It has an increasing incidence in today's world, where the average life expectancy is increasing. Early diagnosis is the most important factor reducing morbidity and mortality, and there is still no marker with high sensitivity and specificity for early diagnosis. In this study, we aimed to find a more sensitive and specific serum marker in the early diagnosis of mesenteric ischemia by comparing thiol with the currently used markers C-reactive protein (CRP) and lactate.

**METHODS:** In our study, 32 Wistar Albino male rats, 10–12 weeks old, weighing 250–300 g, were used. 32 rats were divided into four groups, one of which was the control group. The superior mesenteric artery of the other three groups was ligated. Blood samples were taken after 2 h from the first group, 4 h from the second group, and 6 h from the third group. Then the rats were sacrificed. Mesenteric ischemia and its level were observed in sacrificed subjects. The samples were separated under appropriate conditions and analyzed biochemically.

**RESULTS:** As the ischemia time increased, CRP increased and this increase was found to be statistically insignificant ( $P>0.05$ ). The changes in lactate were found to be statistically significant ( $P<0.05$ ). The difference between the changes of total and native thiol values was found to be statistically significant ( $P<0.05$ ).

**CONCLUSION:** Although CRP is a non-specific parameter in the early diagnosis of acute mesenteric ischemia, lactate maintains its importance as seen in our study. Differences in total thiol and native thiol changes were statistically significant. The fact that this significant difference is observed at the 4th h values reveals the importance of these parameters in early diagnosis. Thanks to the economic and fast results of thiol parameters, it is thought that new studies to be added to the literature can lead to the diagnosis of mesenteric ischemia.

**Keywords:** Early diagnosis; lactate; mesenteric ischemia; thiol.

## INTRODUCTION

Although acute mesenteric ischemia has low incidence of 0.1% of hospital admissions, it is a pathology with mortality ranging between 30% and 90%.<sup>[1]</sup> The most important factor reducing

mortality is early diagnosis but an effective marker for early diagnosis has not been found yet. Although computed angiography is highly sensitive and specific in diagnosis, its role in early diagnosis is quite limited.<sup>[2]</sup>

Early diagnosis in acute mesenteric ischemia reduces both the

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need for bowel resection and the need for open surgery.<sup>[3]</sup> Studies have shown that the first 6-h period from the beginning of the pathology is very important in this early diagnosis, and if this period is exceeded, necrosis may develop and the need for resection may increase.<sup>[4]</sup>

Many imaging and laboratory methods have been tried for the early diagnosis of acute mesenteric ischemia, but a method with sufficient sensitivity and specificity has not been found yet. Today, lactate is used as the most important parameter. However, its sensitivity and specificity alone are not sufficient for diagnosis and especially for early diagnosis.<sup>[5]</sup>

In this study, we planned to carry out a study that may contribute to the clinic in the early diagnosis of mesenteric ischemia using C-reactive protein (CRP) and lactate, which are known to be important in the diagnosis of mesenteric ischemia, and thiol molecules, which we think may be effective in the early diagnosis of mesenteric ischemia due to their biological properties. We aimed to reduce the mortality and morbidity of mesenteric ischemia by finding a new biomarker for early diagnosis with the increase of these molecules over time in rats with mesenteric ischemia.

## MATERIALS AND METHODS

### Experimental Animals and Environment

This study was reviewed by the Local Ethics Committee of KOBAY Experimental Animals Laboratory Inc. and was approved on July 3, 2021 with the decision number 2021/558 in accordance with the ethical committee guidelines. The operations were performed in KOBAY Experimental Animals Laboratory.”

In the study, 32 Wistar Albino male rats, 10–12 weeks old and weighing 250–300 g, were used. The animals were randomly divided into four groups with eight subjects in each group.

Lactate, CRP and thiol changes in serum in blood samples taken after mesenteric ischemia were evaluated in Central Biochemistry Laboratory of Ankara City Hospital.

### Formation of Experimental Groups

32 rats were divided into four groups, each consisting of eight subjects.

- Group 1 Control group (n=8): The group of rats in which mesenteric ischemia was not created, only laparotomy was performed on and blood samples were taken afterwards.
- Group 2 Mesenteric Ischemia 2. h group (n=8): The group whose blood sample was taken 2 h after mesenteric ischemia was created.
- Group 3 Mesenteric Ischemia 4. h group (n=8): The group whose blood sample was taken 4 h after mesenteric ischemia was created.
- Group 4 Mesenteric Ischemia 6. h group (n=8): The group whose blood sample was taken 6 h after mesenteric ischemia was created.

### Surgery Procedure

Rats were anesthetized by applying a single subcutaneous injection of a mixture of xylazine (10 mg/kg) and ketamine (90 ng/kg).

Anesthetized animals were placed on the experimental table in the supine position, fixed by the foreleg and hind legs. To ensure the skin cleanliness of all experimental animals, the skin of the abdominal region was shaved on the operating table and then the skin was cleaned with 10% Povidone iodine. And laparotomy was performed.

Group 1 Control group: After anesthesia, 2.5–3 cm laparotomy was performed. Intestines were observed to be viable, the SMA was confirmed to be open. Blood samples were taken from the subjects into two separate blood tubes and the subjects were sacrificed.

Group 2 Mesenteric Ischemia 2. h group: After anesthesia, 2.5–3 cm laparotomy was performed, SMA was found and clipped. The abdomen was closed and anesthesia continued. At the end of the 2nd h, after the intestinal ischemia and SMA occlusion were confirmed by re-laparotomy, blood samples were taken from the subjects into two separate blood tubes and the subjects were sacrificed. One rat in this group was not included in the study because it died at the 17th min following anesthesia (Fig. 1)

Group 3 Mesenteric Ischemia 4 h group: After anesthesia, 2.5–3 cm laparotomy was performed, SMA was found and clipped. The abdomen was closed and anesthesia continued.



**Figure 1.** Intestinal ischemia after 2 hours of SMA occlusion.



**Figure 2.** Intestinal ischemia after 4 hours of SMA occlusion.

At the end of the 4th h, after the intestinal ischemia and SMA occlusion were confirmed by re-laparotomy, blood samples were taken from the subjects into two separate blood tubes and the subjects were sacrificed (Fig. 2).

**Group 4 Mesenteric Ischemia 6 h group:** After anesthesia, 2.5–3 cm laparotomy was performed, SMA was found and clipped. The abdomen was closed and anesthesia continued. At the end of the 6th h, after the intestinal ischemia and SMA occlusion were confirmed by re-laparotomy, blood samples were taken from the subjects into two separate blood tubes and the subjects were sacrificed (Fig. 3).

#### **Biochemical Analysis**

Blood samples taken with appropriate technique and time were placed into 1 gel biochemistry tube (yellow cap) and 1 EDTA hemogram tube (purple cap). Tubes were centrifuged at 3000 rpm for 10 min. After centrifuging, the serum samples were divided from biochemistry tube into Eppendorf tubes for CRP and Thiol measurements. After the same procedure, sample was taken from the serum obtained from the hemogram tube to the Eppendorf tube for lactate. The samples were kept at +4° and delivered to Central Biochemistry Laboratory of Ankara City Hospital. CRP and Lactate were analyzed in Central Biochemistry Laboratory of Ankara City Hospital. For the thiol tests, the samples were frozen at –80°C first. Then, the samples were examined in Ankara City Hospital Laboratory of Medical Biochemistry Department of Ankara Yıldırım Beyazıt University.



**Figure 3.** Intestinal ischemia after 6 hours of SMA occlusion.

#### **Statistical Analysis**

The study was carried out on 31 subjects. The data were completed by transferring to IBM SPSS Statistics 23 program. While evaluating the study data, descriptive statistics (mean, standard deviation) were given for numerical variables. One-way analysis of variance (One-Way analysis of variance [ANOVA]) was used to determine whether there was a difference between the groups. As a result of the “one-way ANOVA,” the Levene test was first checked for variance homogeneity, and then which group or groups the difference originated from was checked with the “multiple comparison test” (Bonferroni or Tamhane’s T2). The Bonferroni test was used to examine the difference between the groups in the variables that provided variance homogeneity, and the Tamhane’s T2 test was used to examine the difference between the groups in the variables that did not provide the variance homogeneity.  $P < 0.05$  was considered for significance level.

## **RESULTS**

The study was started with 32 rats, eight in each group. However, the study was completed with 31 rats, since in the 2nd h Group I rat died at the 17th min following anesthesia.

#### **CRP**

While there was no significant change in CRP in the 2nd h following ischemia, it increased gradually in 4 and 6 h. While there was an increase in the 4th and 6th h of ischemia, this

**Table 1.** Examining the differences in CRP values between groups

	Average	SD	F	P-value	Difference
1. Control group	0.19	0.06	2.269	0.103	-
2. Group with mesenteric ischemia blood taken at the 2nd h	0.19	0.07			
3. Group with mesenteric ischemia blood taken at the 4th h	0.24	0.11			
4. Group with mesenteric ischemia blood taken at the 6th h	0.28	0.07			
Total	0.22	0.08			

F: One-way ANOVA testi<sup>†</sup>: P<0.05.**Table 2.** Examining the differences in lactate values between groups

	Average	SD	F	P-value	Difference
1. Control group	15.76	1.56	226.899	0.000*	1<2<3<4
2. Group with mesenteric ischemia blood taken at the 2nd h	25.14	2.96			
3. Group with mesenteric ischemia blood taken at the 4th h	46.31	7.81			
4. Group with mesenteric ischemia blood taken at the 6th h	68.75	1.91			
Total	39.44	21.36			

F: One-way ANOVA testi<sup>†</sup>: P<0.05.**Table 3.** Examination of the differences in native thiol values between groups

	Average	SD	F	P-value	Difference
1. Control group	376.33	47.87	4.877	0.008*	1>3
2. Group with mesenteric ischemia blood taken at the 2nd h	340.96	84.84			
3. Group with mesenteric ischemia blood taken at the 4th h	239.98	57.42			
4. Group with mesenteric ischemia blood taken at the 6th h	296.66	99.75			
Total	312.59	88.38			

F: One-way ANOVA testi<sup>†</sup>: P<0.05.

increase was found to be statistically insignificant (P>0.05) Table 1.

### Lactate

The amount of lactate increased in correlation with the ischemia time. Compared to the control group, it increased approximately 3 times in the 4th h group and approximately 4 times in the 6th h group. When these increases were analyzed statistically, they were found to be statistically significant (P<0.05). (Table 2)

### Native Thiol

Native thiol value decreased gradually in the 2nd and 4th h groups compared to the control group; however, it increased in the 6th h group. Despite this increase, the value in the 6th h group did not reach the value of the 2nd h group. When these changes were examined statistically, the change in the

4th h group was found to be statistically significant (P<0.05). (Table 3)

### Total Thiol

Total thiol value decreased gradually in the 2nd and 4th h groups compared to the control group; however, it increased in the 6th h group. Despite this increase, the value in the 6th h group did not reach the value of the 2nd h group. When these changes were examined statistically, the change in the 4th h group was found to be statistically significant (P<0.05). (Table 4)

## DISCUSSION

In this study, the use of new biomarkers in the early diagnosis of mesenteric ischemia is discussed. This is the only animal study in the literature comparing CRP, lactate and thiol parameters and showing that thiol is significant in early diagnosis.

**Table 4.** Examination of the differences in total thiol values between groups

	Average	SD	F	P-value	Difference
1. Control group	413.60	51.52	6.639	0.002*	1.2>3
2. Group with mesenteric ischemia blood taken at the 2nd h	384.95	78.92			
3. Group with mesenteric ischemia blood taken at the 4th h	276.20	52.20			
4. Group with mesenteric ischemia blood taken at the 6th h	339.53	75.98			
Total	352.56	81.77			

F: One-way ANOVA testi: P&lt;0.05

Acute mesenteric ischemia is still an important health problem as a pathology with mortality of 30–90%, and its incidence is increasing.<sup>[1]</sup> This pathology is associated with serious morbidity in patients. The most important of them is short bowel syndrome. Patients may experience severe diarrhea due to this and may encounter a mortal malabsorption situation.<sup>[6]</sup>

The most important reason for the high mortality and morbidity in acute mesenteric ischemia is the absence of any test or physical examination finding with high sensitivity and specificity in the diagnosis. Accordingly, delay in diagnosis and treatment increases the mortality and morbidity of the disease. Thanks to early diagnosis, mucosal injury may be reduced, especially with endovascular interventions, with methods that restore blood circulation, without tissue and organ loss. And it decreases the mortality and morbidity of the disease.<sup>[7]</sup>

Although in studies many markers have been found to be beneficial in early diagnosis, their sensitivity and specificity are still limited.

In our study, in accordance with the literature, lactate is found to be statistically significant in the diagnosis of mesenteric ischemia, but in line with our hypothesis, also total thiol and native thiol from thiol-disulfide parameters are found to be statistically significant in the diagnosis. And it is showed that, in addition to the literature, total thiol and native thiol may be used in the early diagnosis of mesenteric ischemia.

Studies have shown that CRP is elevated with mucosal injury in acute mesenteric ischemia. However, it is stated that this increase is not significant enough for diagnosis when compared with the control group. Especially the increase of CRP in other inflammatory processes reduces its specificity in the diagnosis of mesenteric ischemia.<sup>[8]</sup>

One of the parameters was taken as CRP in the study of Ozcay N. and friends by creating partial and diffuse mesenteric ischemia in an animal model. Although there was an increase in CRP value, no significant difference was observed between the two groups in terms of CRP. And it is showed that the help of CRP is limited even in the extent of the pathology.<sup>[9]</sup>

In our study, the place of CRP in diagnosis is similar to that in

the literature. In our animal experiment, it was observed that CRP increased as ischemia time progressed. Especially at the 4th and 6th h, the increase in CRP continued. However, as in the literature, this increase was not found to be statistically significant in our study either.

Lactate is a parameter that still maintains its importance in the diagnosis of mesenteric ischemia. Murray et al. compared the amount of lactate in mesenteric ischemia with ileus and other acute abdomen diagnoses that might be confused with mesenteric ischemia and made a very important contribution to the literature in this field.<sup>[10]</sup> In a study conducted with pigs, it was determined that the amount of lactate in the venous blood increased significantly with SMA occlusion, and its role in early diagnosis gained importance.<sup>[11]</sup> The fact that lactate increases with the duration of ischemia and reaches very high values with intestinal necrosis, reveals its importance in early diagnosis.<sup>[12]</sup> In another study conducted on rats, the increase in lactate was found to be statistically significant in the diagnosis of mesenteric ischemia.<sup>[13]</sup>

However, its increase in all kinds of ischemia limits the role of lactate in the diagnosis of mesenteric ischemia. Because in patients with mesenteric ischemia, the main pathology develops in the cardiovascular system, similar pathologies may affect all kinds of vascular structures, and ischemia due to embolism and thrombosis may occur in many organs. As a result, lactate increases and the specificity of the lactate parameter to mesenteric ischemia decreases.<sup>[14]</sup> However, lactate is still the most important data available for comparison with other parameters. In many studies in the literature comparison with lactate is made to find a new marker.<sup>[15]</sup>

In our study, the amount of lactate was found to be high in models with mesenteric ischemia, as in the literature. Furthermore, again as in the literature, as the duration of ischemia increased, the amount of lactate was found to be higher in the blood of rats with ischemia. Lactate values were found to be statistically significant as a result of the statistical analysis.

In recent years, free radicals have been accused as important etiological causes of inflammatory pathologies, ischemia and even cancers at the cellular level. It is thought that the main pathology is caused by the disorder of the oxidant-antioxi-

dant balance and many parameters have been investigated in many pathologies. Thiol-disulfide homeostasis is one of these parameters.<sup>[16]</sup>

In a study conducted with rats, it was stated that total and native thiol amounts decreased and disulfide amounts increased at the 3rd and 6th h by creating mesenteric ischemia, and it was reported that it is a parameter to be used with IMA.<sup>[13]</sup> In another animal experimental model, no statistically significant difference was found.<sup>[17]</sup>

In our study, the amount of native thiol and total thiol decreased in the 2nd and 4th h ischemia groups, but increased in the 6th h ischemia group, similar to the literature. These changes were found to be statistically significant in statistical analysis. The mean amount of disulfide in the groups varied independently of ischemia and ischemia duration. Therefore, there was no statistically significant difference.

## CONCLUSION

Although CRP is not widely used clinically, it has been found to be statistically insignificant in our study. In our study, lactate has once again proved the importance of its place in clinical use. However, these two parameters limit their place in early diagnosis, as they constantly increase as ischemia continues and do not give any cut-off value at any time. The fact that the total thiol and native thiol changes are found to be statistically significant, especially the 4th h values, show that they can be involved in the early diagnosis of mesenteric ischemia. In addition, this result is the first and only result in animal experiments showing that thiol parameters may be significant for the diagnosis of mesenteric ischemia in the literature. Thanks to the fact that these parameters may be measured rapidly and unexpensively today, it is thought that they may be the light at the end of the tunnel in the early diagnosis of mesenteric ischemia with additional studies in the literature. Animal models are important because of their experimental nature. However, they may not fully cover outcomes in humans. Therefore, clinical studies on this subject are also needed.

**Ethics Committee Approval:** This study was approved by KOBAY Research Laboratory Ethics Committee (Date: 03.07.2021, Decision No: 558).

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**Conflict of Interest:** None declared.

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DENEYSEL ÇALIŞMA - ÖZ

## Mezenter iskeminin erken tanısında thiol, CRP ve laktat'ın öneminin araştırılması: Hayvan deneyi modeli

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**AMAÇ:** Akut mezenterik iskemi özellikle yaşlı popülasyonda görülmektedir. Ortalama yaşam ömrünün arttığı günümüz dünyasında giderek artan bir insidansa sahiptir. Erken tanı morbidite ve mortaliteyi azaltan en önemli etken olup, erken tanıyı sağlamada sensitivitesi ve spesifitesi yüksek bir belirteç halen bulunmamaktadır. Bu çalışmada, thiol ile mevcut aktif kullanılan belirteçler olan C reaktif protein ve laktat ile karşılaştırarak mezenterik iskemi erken tanısında daha sensitif ve spesifik bir serum belirteci bulmayı amaçladık.

**GEREÇ VE YÖNTEM:** Çalışmamızda 32 adet 10-12 haftalık 250-300 gr ağırlığında Wistar Albino erkek sıçan kullanıldı. 32 sıçan, bir tanesi kontrol olmak üzere 4 gruba ayrıldı. Diğer 3 grubun superior mezenter arteri bağlandı. Bir gruptan 2, ikinci gruptan 4, üçüncü gruptan 6 saat sonra kan örneği alınarak sıçanlar sakrifiye edildi. Sakrifiye edilen deneklerde mezenter iskemi ve düzeyi gözlemlendi. Alınan örnekler uygun koşullarda ayrıştırılarak biyokimyasal olarak analiz edildi.

**BULGULAR:** CRP iskemi süresi arttıkça yükselmekle beraber bu yükseliş istatistiksel olarak anlamsız bulunmuştur ( $p>0.05$ ). Laktattaki değişimler ise istatistiksel olarak anlamlı bulunmuştur ( $p<0.05$ ). Total ve native thiol değerlerinin değişimleri arasındaki fark istatistiksel olarak anlamlı bulunmuştur ( $p<0.05$ ).

**SONUÇ:** Akut mezenterik iskeminin erken tanısında CRP non spesifik bir parametre olmakla beraber laktat çalışmamızda da görüldüğü gibi önemini korumaktadır. Total thiol ve native thiol değişim farkları istatistiksel olarak anlamlı bulunmuştur. Bu anlamlı farkın kendini 4. saat değerlerinde göstermesi erken tanıda bu parametrelerin önemini gözler önüne sermektedir. Thiol parametrelerinin ucuz ve hızlı sonuçlanması sayesinde literatüre eklenecek yeni çalışmalar ile mezenter iskemi tanısında yol alınabileceği düşünülmektedir.

**Anahtar sözcükler:** Mezenter iskemi; thiol; laktat; erken tanı.

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