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ARAŞTIRMA / RESEARCH

The role of mean platelet volume and ischemia modified albumin levels in early periods of acute mesenteric ischemia: an experimental study

Akut mesenterik iskeminin erken dönemlerinde ortalama platelet hacmi ve iskemi modifiye albümin düzeylerinin rolü: bir deneysel çalışma

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Abstract

Purpose: The aim of this study was to determine the role of mean platelet volume (MPV) and ischemia modified albumin (IMA) in early diagnosis of acute mesenteric ischemia (AMI).

Materials and Methods: In this study, 18 Wistar Albino rats were randomly assigned to 3 experimental groups with 6 rats in each group as; sham group without laparotomy, control group with laparotomy and mesenteric ischemia-induced group. Platelet count, MPV and IMA were studied at the end of the 120th min of superior mesenteric artery obstruction in AMI group and at the same time in other groups.

Results: In AMI group MPV and IMA values were significantly higher compared with other 2 groups, while platelet count was significantly lower . There was not any statistically significant difference between control and sham groups regarding these parameters.

Conclusion: Further clinical studies are required to define the role of these parameters in early diagnosis of AMI since AMI is a highly mortal disease if not diagnosed promptly

Keywords: Acute mesenteric ischemia, mean platelet volume, ischemia modified albumin.

Öz

Amaç: Bu çalışmanın amacı akut mezenterik sikeminin (AMİ) erken tanısında ortalama trombosit hacminin (MPV) ve iskemi modifiye albümin (IMA) dğzeylerinin rolünü belirlemekti.

Gereç ve Yöntem: Bu çalışmada, 18 Wistar Albino cinsi sıçan, her grupta 6 sıçan olacak şekilde 3 deney grubuna rasgele olarak ayrıldı; laparotomisiz şam grubu, laparotomi yapılan control grubu ve mezenterik iskemi grubu. Trombosit sayısı, MPV ve IMA, AMI grubunda 120. dk.lık superior mezenterik arter tıkanıklığının sonunda ve aynı zamanda diğer gruplarda çalışıldı.

Bulgular: AMI grubunda MPV ve IMA değerleri diğer 2 gruba göre anlamlı derecede yüksekti; trombosit sayısı anlamlı olarak düşüktü. Kontrol ve şam grupları arasında bu parametreler açısından istatistiksel olarak anlamlı bir fark yoktu.

Sonuç: AMI hızlı tanı konulmazsa oldukça mortal olabilen bir hastalıktır. Bu nedenle erken tanıda bu parametrelerin rolünü tanımlamak için daha fazla klinik çalışma gereklidir.

Anahtar kelimeler:. Akut mezenter iskemi, ortalama trombosit hacmi, iskemi modifiye albumin.

INTRODUCTION

Acute mesenteric ischemia (AMI) is a rare gastrointestinal emergency that is defined as a sudden decline on the blood supply to a segment of the small intestine causing ischemia and necrosis[1]. AMI may lead to life threatening complications such as bowel infarction, perforation, or peritonitis if not diagnosed promptly and the mortality rates may be as high as 50-80%[2,3]. The main risk factors associated with AMI are dysrhythmia including atrial fibrillation; some cardiac diseases such as endocarditis or cardiac failure; pancreatitis, some hormonal treatments and multi- organ dysfunction[4].

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By the way of recent advances in treatment with endovascular techniques such as percutaneous transluminal angioplasty and stenting, the use of intra-arterial thrombolysis, vasodilators and suction embolectomy; the mortality rates due to AMI become to decrease in patients especially diagnosed at early periods[5,6].

Today, contrast-enhanced computed tomography (CECT) is the mainstay in diagnosis of AMI[7]. But it has been reported that more than half of the AMI cases cannot be diagnosed with CECT if there is no clinical suspicion[8]. Unfortunately, although many blood tests such as D-dimer, transaminases, and creatinine phosphokinase were suggested for AMI diagnosis; none of them were defined as sufficient in terms of sensitivity and specificity[9,10].

In about 80% of AMI cases, thromboembolic diseases play an important role. In circulation, there are many platelets with different size and density. The larger platelets are known to be more active and having more thrombotic features due to their dense granules[11]. Platelet volume, which is defined with mean platelet volume (MPV) in blood tests, is a marker of platelet activation[12]. For that reason, MPV is expected to be increased in thrombogenic disease including AMI. In acute ischemic conditions, metal binding capacity of albumin is reduced and a metabolic variant of albumin is produced, called as ischemia modified albumin (IMA)[13]. In an ischemic environment, due to the structural change of NH_2, the ability of albumin to bind cobalt is decreased and IMA is produced.

Elevated IMA levels in some ischemic conditions and in some diseases associated with chronic oxidative stress including myocardial ischemia, preeclampsia, diabetes mellitus and pancreatitis has been reported before[14-16]. In this experimental study, we aimed to determine the role of mean platelet volume and ischemia modified albumin in early diagnosis of AMI. In previous literature there are a few studies about the role of MPV and/or IMA in diagnosis of AMI but the data is still limited.

MATERIALS AND METHODS

This study was approved by Antalya Education and Research hospital ethics committee and the study was performed in Akdeniz University Medical Faculty Experimental Animals Laboratory (Ethical approval number: 03-09-2012/ 62). In this study, 18 Wistar Albino rats, that were 2-4 months old with a weight of 180-250 gr, were used. The rats were fed with city water and standard feed in appropriate cages prior to the experiment. Animals were fed 10 days prior to the experiment without water restriction in individual cages, and were fed with standard feed to comply with ambient conditions. The rats were housed in a constant temperature, with 12 hours dark and 12 hours light cycles. The animals were randomly assigned to 3 experimental groups with 6 rats in each group and placed in each cage as one rat. Group A (n=6) is composed of sham group without laparotomy, Group B (n=6) as control group with laparotomy and Group C (n=6): is composed of mesenteric ischemia-induced group.

Surgical procedure

The rats were fasted 12 hours before the operation but the water intake was not restricted. The rats were anesthetized with ether, first. Then for prolonged anesthesia and analgesia 50 mg /kg ketamine hydrochloride (Ketalar®, Eczacibasi, Istanbul, Turkey), and 10 mg / kg xylazine HCl (Alfazyne® 2% 20 mg / mL, 30 mL, Alfasan Int BV. Netherlands) was injected intramuscularly.

Midline of the abdomen of the rats is cleaned down with 10% Povidone Iodide (Batticon® solution, Adeka, Turkey) for the skin antiseption. Following sterile sheathing, the abdomen was inserted through the intraabdominal cavity with a 3 cm incision in the middle. All small intestines were taken out.

In the mesenteric ischemia-generated group, atraumatic microvascular clamp was immediately placed just to the distal site of the SMA branching from the aorta. After 120 min clamping, making sure that the pulse was lost, ischemia was observed in the intestines. At the end of this period, intracardiac blood was taken from the right ventricle for evaluations. Blood obtained was centrifuged and stored at -80 ° C until the day of study. Platelet count, mean platelet volume and ischemia modified albumin were measured from the samples. Any of the rats were not lost during the experiment. At the end of the surgical procedure, all subjects were terminated by cervical dislocation.

Measurements

The alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels in serum were measured with a commercial kit using an auto

analyzer (Sysmex America, Inc., Mundelein, IL, USA). Veterinary automated cell counter Sysmex WE-2100 America (Sysmex America, Inc., Mundelein, IL, USA) was used to analyze the platelet count, and mean platelet volume. Ischemia Modified Albumin level was analyzed using the rapid and colorimetric method described by Bar-Or et al (13). In summary 200 µL rat serum was mixed with 50 µL of 0.1% $CoCl_2$•$6H_2O$ (Sigma, St. Louis, MO) in a glass tube and gently shaken. After 10 min, 50 µL of dithiothreitol (DTT; Sigma, 1.5 mg/mL H_2O) was added. Then after 2 min, 1.0 mL 0.9% NaCl was added to extinguish the reaction. After colorimetric analysis at 470 nm using a spectrophotometer (Model UV1601, Shimadzu, Sydney, Australia), the results were reported as absorbance units (ABSUs).

Statistical analysis

Mean ± standard deviation, median and min-max values are used in the descriptive statistics of the data. The distribution of the variables was checked by the Kolmogorov Simirnov test. ANOVA was used for analysis of quantitative data, and Tamhane test was used for sub-analyzes. SPSS 21.0 program was used in the analyses.

RESULTS

All rats completed the study without any lost. The results of blood analyses are summarized in Table 1. In group C; AST, ALT, MPV and IMA values were higher compared with groups A and B ($p < 0.05$); while in group C, platelet count was significantly lower than groups A and B ($p < 0.05$). AST, ALT, MPV, IMA, and platelet values were not significantly different between groups A and B ($p > 0.05$) (Figures 1-3).

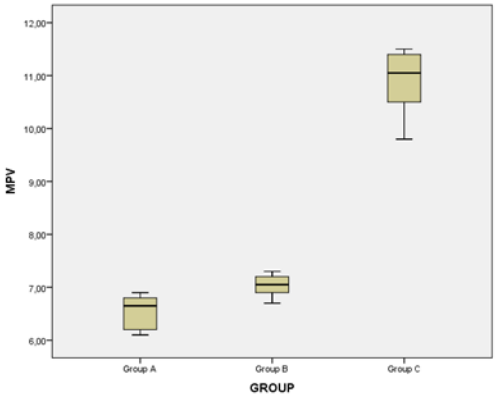

Figure 1. Distribution of MPV among groups.

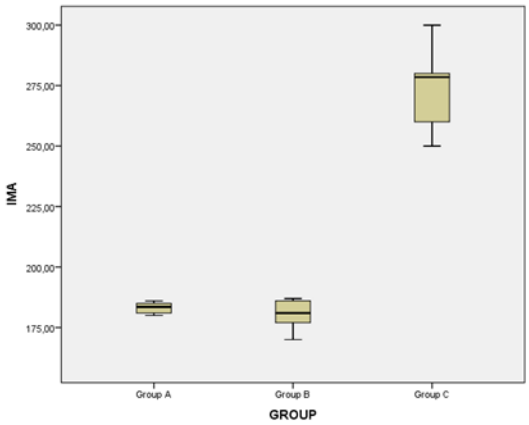

Figure 2. Distribution of IMA among groups

Table-1. Results of biochemical analyses among groups

Mean ± SD Median (Range)	Group A (n:6)	Group B (n:6)	Group C (n:6)	p
AST (IU/l)	33.5 ±2.4* 34 (30-37)	33.5 ±3.0* 34 (30-37)	63.8±9.0 64 (50-75)	0.001
ALT (IU/l)	26.7±2.2* 27 (24-30)	26.0±3.7* 27 (21-30)	51.3±10.6 52 (39-65)	0.001
MPV (µl)	6.6±0.3* 7 (6-7)	7.0 ±0.2* 7 (7-7)	10.9 ±0.6 11 (10-12)	0.001
IMA	183.2± 2.3* 184 (180-186)	180.3 ±6.3* 181 (170-187)	274.5 ± 17.5 279 (250-300)	0.001
Thrombocyte ($x10^3$)	107.1± 6.3* 106.5 (98-117)	102.6± 13.2* 96.5 (90-120)	33.8± 5.7 35.5 (25-40)	0.001

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, MPV:Mean platelet volume, IMA: Ischemia modified albumin. ANOVA/Tamhane/ *:statistically significantly different when compared with group C, $p<0.05$.

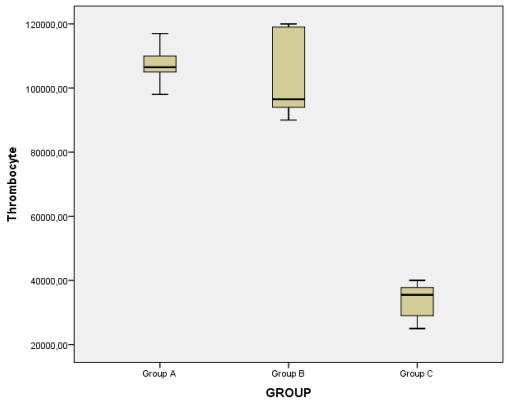

Figure 3. Distribution of thrombocyte counts among groups

DISCUSSION

In this experimental study, we analyzed the alterations in serum MPV and IMA levels and thrombocyte count in acute mesenteric ischemia and we determined that; at the end of 120 minutes, in acute mesenteric ischemia, serum MPV and IMA levels increase while platelet count decrease significantly. Since early diagnosis is life-saving in this urgent condition these parameters may be promising in prompt diagnosis of AMI.

Although rare, AMI is a highly mortal disease with its acute complications. The main factor responsible from the high mortality rates is the delay in diagnosis[3]. In clinical practice an acute onset abdominal pain is the most common symptom which is highly non-specific[17]. Moreover although many blood tests were studied before in early diagnosis of AMI, none of them were reported to have enough sensitivity or specificity for diagnosis[10]. Due to nonspecific clinical and laboratory findings, diagnosis of AMI becomes delayed which is an important factor increasing mortality. The mortality rates associated with AMI have been reported as high as 30-85% in previous literature[18,19]. Regarding these reasons, it is important and essential to define a rapid, less invasive and simple method in diagnosis of AMI[20].

Mean platelet volume is known to be an indicator of platelet activation that is increased in thrombogenic diseases. It is also a simple parameter that can routinely be obtained in all laboratories, inexpensive and easy to interpret. In previous literature, there are a few studies investigating the role of MPV in diagnosis of AMI. Supporting our results, in a retrospective study, Turkoglu et al[21] reported that MPV values were significantly higher in patients with AMI than in the healthy controls. In a case control study, Degerli et al[22] reported significantly higher MPV levels in AMI group compared with healthy controls. But when the patients were compared with their matched controls for concomitant diseases there was not any significant difference in MPV levels suggesting that MPV may be used as an indicator of AMI only in patients without any concomitant diseases. Recently, in a retrospective clinical study Wang et al[23] reported higher MPV levels in patients with AMI, compared with other acute abdomen cases and they suggested a diagnostic score with high sensitivity and specificity including MPV levels. Recently, MPV was also suggested as a prognostic factor in AMI[24,25]. Similarly, in a systematic review, Budak et al[26] investigated the role of platelet indices in non-traumatic abdominal surgery and reported that high MPV levels were associated with poor prognosis in AMI. In the light of our results and previous literature, elevated MPV values may be suggested as n early diagnostic marker in AMI.

A decrease in platelet count determined in AMI group in this study may be associated with the excessive consumption of platelets[27]. However, in contrast with our results Toptas et al[28] did not determine any significant alterations in platelet counts between patients with AMI and age and sex-matched healthy controls. Platelet count not itself only, but MPV that is showing the platelet activity, may be more appropriate as a diagnostic marker of AMI.

Ischemia modified albumin is another biomarker, that was associated with ischemic conditions. First in year 2008, in thromboembolic occlusion of the superior mesenteric artery, IMA levels were reported to be statistically significantly higher compared with the control cases[29]. Later Gunduz et al[30] reported that plasma IMA levels in the AMI groups were significantly higher compared to those of the control groups while IMA levels increased significantly at the end of 2-hour compared with the 30-minute results and at the end of 6-hour compared with the 2 hour values. In an experimental study, Dundar et al[31] reported that the serum IMA levels of the ischemia group at hours 3

and 6 were significantly higher than those of the control and sham groups. In an experimental study, Kadioglu et al[32] reported that IMA may be suggested as an effective marker in incarcerated hernias to predict necrosis. Supporting these data, Treskes et al[33] reported that, the novel serological biomarkers including IMA may offer improved diagnostic accuracy of AMI. Our results were also compatible with the previous literature in general as defining elevated IMA levels at the end of the 2nd hour in AMI. However, in contrast with all those findings, Uygun et al[34] compared the IMA levels in 4 rat groups (control, sham, 2- and 6-hour ischemia groups) and did not determine any significant alterations regarding IMA levels in those 4 groups; although the histopathological evaluation of the intestinal wall of those groups were different from each other. We also determined elevated ALT and AST levels in AMI. Similar with our results Zhang et al[35] also reported elevated ALT and AST levels but later, on 6th and 9th hours of superior mesenteric vein ligation. However, since liver function tests may be elevated in many conditions associated with acute abdomen, they, alone, may not be valuable in early diagnosis of AMI. In previous literature, ALT and AST were also suggested as prognostic indices in AMI[36].

There are some limitations of this study that should be mentioned. This is an animal study carrying all bias associated with the differences in animal and human metabolisms. Second, we measured the serum levels of MPV and IMA once at 2nd hour. However, alterations of these parameters at different time points may also be important. And lastly, AMI is a disease most commonly seen in elderly and in patients with many co-morbid conditions which may also cause alterations in clinical practice.

In conclusion, in this study we determined that, at the end of the second hour of AMI, MPV and IMA levels increased significantly while platelet count significantly decreased. Further clinical studies are required to define the role of these parameters in early diagnosis of AMI since AMI is a highly mortal disease if not diagnosed promptly.

Yazar Katkıları: Çalışma konsepti/Tasarımı: NB; Veri toplama: GC; Veri analizi ve yorumlama: TB; Yazı taslağı: GC, TB; İçeriğin eleştirel incelenmesi: NB; Son onay ve sorumluluk: GC, TB, NB; Teknik ve malzeme desteği: TB; Süpervizyon: NB; Fon sağlama (mevcut ise): yok.
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