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Values of troponin T and myoglobin predictive of non-cardiac ischemia in rats

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ABSTRACT

Objective(s): Biochemical markers are important for the timely diagnosis and follow-up of ischemic events. Most of the markers have been previously studied in the context of cardiac ischemia. However, research on markers of non-cardiac events has been insufficient. Therefore, we investigated the relationship between troponin and myoglobin which are commonly used markers of cardiac ischemia, in non-cardiac ischemia.

Materials and Methods: Forty-eight rats were equally divided into six groups. Group I was the control group. Group II was the sham group and received a simple laparotomy. The superior mesenteric artery was clamped in groups III and IV in order to create mesenteric ischemia. The left femoral artery was clamped in groups V and VI in order to create peripheral ischemia. Intracardiac blood samples were taken from all groups (during the 3rd hour of ischemia in groups III and V and the 6th hour of ischemia in group IV and VI) and troponin T and myoglobin levels were measured.

Results: Troponin and myoglobin levels were statistically similar in groups I and II. Moreover, increments were detected for troponin and myoglobin in ischemia groups according to group I and II. Furthermore, higher troponin Tlevels were detected after three hours of mesenteric ischemia and higher myoglobin values were observed after six hours of mesenteric ischemia (P<0.05).

Conclusion: Troponin T and myoglobin are not specific for non-cardiac ischemia, and they may be useful for detecting other ischemic events.

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Introduction

Ischemia is defined as inadequate delivery of oxygen to cells of different parts of the body, such as the intestines and limbs. It can be acute, chronic or acute superimposed on chronic (1). Biochemical markers may be released during the ischemic period. These markers (e.g. creatinine kinase, myoglobin and troponins) have been used to diagnose cardiac ischemia in clinical practice (2). Previous studies have shown that troponin T and myoglobin have excellent sensitivity for the diagnosis of acute myocardial infarction, but currently no available plasma biomarker has been proven to be enough specific to serve as an early diagnostic test for non-cardiac ischemia (2, 3). There are few studies on the significance of these markers in non-cardiac ischemia (4). Myoglobin is a cytoplasmic heme-containing protein which is produced in cardiomyocytes and skeletal myocytes. It functions as an intracellular oxygen reservoir in muscle cells and expedites the supply of oxygen during ischemia and expedites the supply of oxygen during ischemia or exercise (5). Troponins are heteromeric proteins that play a significant role in the regulation of skeletal and cardiac muscle contraction. This family includes troponins I, T and C. Troponins are released into the circulation and may be detected in the peripheral blood 3-6 hr after an ischemic event (6). Previous studies suggested that troponin T can play an important role in risk stratification of non cardiac events via various mechanisms (6).

The aim of this study was to investigate the levels and the clinical significance of troponin T and myoglobin levels in rats with non-cardiac ischemia. Troponin T and myoglobin were chosen for this study to clarify suggestions that have been mentioned in recent studies.

Materials and Methods

Study design

This animal study was designed as a rando-mized, interventional animal study. The local animal

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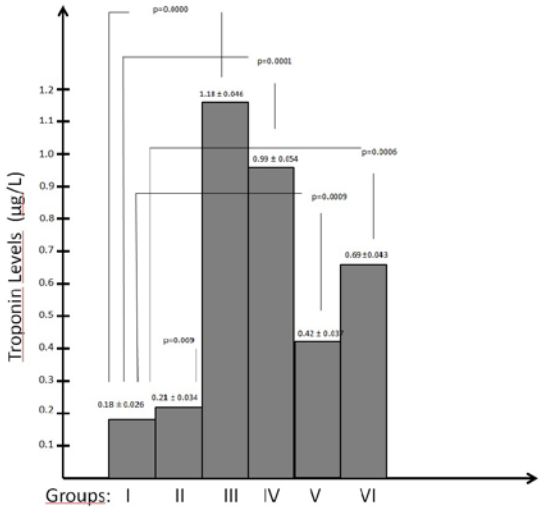

Figure 1. Comparison of troponin t levels among all groups
Group I: Control group, group II: Sham group, group III: Three hr after mesenteric ischemia, group IV: Six hr after mesenteric ischemia, group V: Three hr after peripheral ischemia and group VI: Six hr after peripheral ischemia. *P*<0.05 is considered significant

ethics committee approved the protocol. Healthy male Wistar albino rats with an average weight of 250-300 g were procured from the Laboratory Animal Production Unit of Dicle University.

Animal subjects

Forty-eight rats were used in the experiments. The experiments were performed in laboratories with standardized conditions at 22±2°C and 50±5% humidity. A standard diet and tap water were provided *ad libitum*. Twelve hours prior to the experimental procedure, the rats were given only water.

Study protocol

Rats were divided into six randomized equal groups. Ketamine 130 mg/kg (Ketalar, Pfizer) and xylasine 20 mg/kg (Rompun, Bayer) were used to induce anesthesia. Additionally, ketamine HCl (50 mg/kg) was used during the surgeries for anesthesia maintenance. All vital signs (respiratory rate, pulse, oxygen saturation (So_2), and body temperature) were monitored.

The control group (Group I) was employed to determine the basal troponin and myoglobin levels in rats. From group I, blood samples were taken but no surgeries were performed. A sham group (Group II) was used to determine the impact of surgical incision on biochemical parameters and blood samples were taken following a simple laparotomy. Two mesenteric ischemia groups were created (group III-IV) by ligating the superior mesenteric artery (SMA) via a simple laparatomy. Intracardiac blood samples were taken 3 hr after SMA ligation in group III and 6 hr after ligation in group IV. In order to create peripheral ischemia, the left femoral artery was clamped in two groups of rats (group V-VI). Intracardiac blood samples were prepared after 3 hr of ischemia in group IV and after 6 hr in Group VI. After the experimental procedures, all rats were sacrificed.

Laboratory analysis

Point-of-care testing (POCT) by Roche (*Roche* Diagnostics, Mannheim, Germany) was performed on each whole blood sample. The samples were obtained in vacuum tubes (which contained separating gel and lithium heparin) and troponin T and myoglobin levels were quantified simultaneously using a fluorescence immunoassay.

Troponin-T and myoglobin measurement

Myoglobin and troponin T levels were measured in whole blood by POCT using the Roche Cobas® h 232 immunoassay analyzer (*Roche* Diagnostics, Mannheim, Germany). The range of detection was 0.1 to 3 µg/l for troponin T and 30 to 700 µg/l for myoglobin (7, 8).

Statistical analysis

The results were expressed as mean±standard deviation (SD). The Kolmogorov-Smirnov test was used to determine if the data were normally distributed. Analysis of variance (ANOVA) and Bonferroni post-hoc tests were used to compare the groups. All statistical procedures were performed using SPSS software version 15.0 (SPSS Inc., Chicago, IL). A *P*-value of ≤0.05 was considered statistically significant.

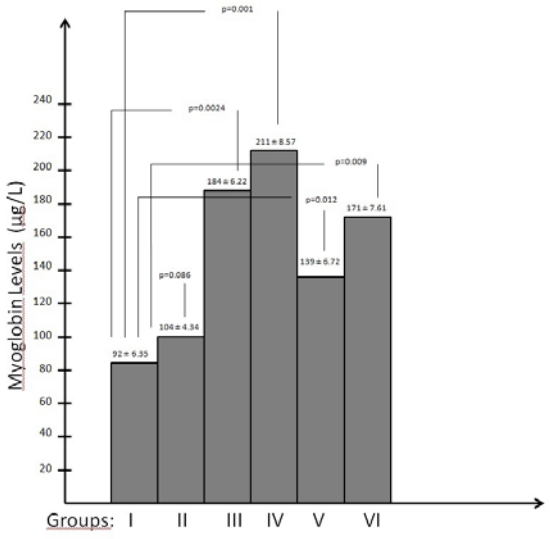

Figure 2. Comparison of myoglobin levels among all groups
Group I: Control group, group II: Sham group, group III: Three hr after mesenteric ischemia, group IV: Six hr after mesenteric ischemia, group V: Three hr after peripheral ischemia and group VI: six hr after peripheral ischemia. *P*<0.05 is considered significant

Results

The mean troponin T levels in groups I, II, III, IV, V, and VI were found to be 0.18±0.026, 0.21±0.034, 1.18±0.046, 0.99±0.054, 0.42±0.037, and 0.69±0.043, respectively (Figure 1). The troponin T levels were significantly higher in the study groups compared to the control group (P<0.05). Moreover, 3 hr after SMA ligation, the mean troponin T level was higher than any other study groups (Group III). A comparison of the mean troponin T values using ANOVA and *post-hoc* Bonferroni tests is presented in Figure 1.

The mean levels of myoglobin in groups I, II, III, IV, V, and VI were found to be 92±6.35, 104±4.34, 184±6.22, 211±8.57, 139±6.72 and 171±7.61, respectively (Figure 2). The myoglobin levels were significantly higher in study groups compared to the control group (P<0.05). Furthermore, higher levels were observed in the mesenteric ischemia groups at the 3rd and 6th hours (Group III-IV). Figure 2 shows the myoglobin values for groups following the statistical analysis using ANOVA and *post-hoc* Bonferroni tests.

As shown in Figures 1 and 2, troponin T and myoglobin have similar levels in both peripheral and mesenteric ischemia in the first six hours after the onset of ischemia. Nevertheless, the highest mean troponin T level was observed 3 hours after the onset of mesenteric ischemia. Six hours after the onset of mesenteric ischemia, the mean troponin T level (Group IV) was lower than its level at 3 hours (Group III).

Discussion

Myoglobin is an essential striated muscle O_2 storage protein that was demonstrated to be highly reactive with peroxidate in the presence of H_2O_2 mediated oxidation (9). It is responsible for the release of O_2 during periods of hypoxia and anoxia. Therefore, myoglobin was investigated as a marker of ischemic muscle (9, 10). A study by van Weel *et al* suggested that myoglobin may lead to improved vascularization in patients with arterial obstructive disease who were treated with therapies designed to increase angiogenesis (5). These results suggest that myoglobin is an important protein in the setting of muscle tissue hypoxia and reorganization. Troponin is a contractile regulatory muscle protein complex that binds to calcium in the actin-myosin bundles within muscle fibers. There are three types of troponin, classified as troponin C, T, and I. The C type is associated with striated muscles. However, troponin I and T are closely associated with cardiac muscles (11). Although troponin I and T are specific for cardiac muscle, significant changes in both of these troponin subtypes have been reported during ischemia of other organs (12). Our findings support the idea that myoglobin and troponin T levels are increased in both mesenteric and peripheral ischemia.

Troponin T levels were investigated in patients undergoing surgery for an abdominal aortic aneurysm or lower extremity arterial obstruction (13). The authors suggested that elevated troponin T levels may predict late mortality. In another study, it was found that troponin T was increased in patients with acute limb ischemia. Additionally, in that study it was suggested that troponin T levels may be an early marker of overall disease severity and a predictor of outcome (14). Also, it was reported that elevated troponin I is a common finding in patients with acute mesenteric ischemia (3). Myoglobin has also been investigated as a biomarker in ischemic tissues, especially in ischemic muscles (9, 10). Although the specific role of myoglobin in hypoxia is unclear, previous reports have suggested that hypoxia regulates myoglobin expression and myoglobin prodution is an adaptive response to hypoxic conditions (15, 16). Yang *et al* studied myoglobin expression in an experimental hindlimb ischemia model in mice. They concluded that elevated myoglobin expression in ischemic skeletal muscle reduces the endogenous perfusion recovery (16). The role of vascular myoglobin in hypoxic conditions was studied and it was suggested that myoglobin contributes to nitrite-induced vasodilation in these conditions, significantly (17). In this study, we created experimental acute mesenteric and peripheral ischemia by clamping sustaining arteries and then drew blood samples three and six hr after the ischemic event. Troponin T and myoglobin values were measured in these blood samples. We found that these two markers were significantly elevated (P<0.05) in both peripheral and mesenteric ischemia. The highest troponin T levels were observed in the samples taken three hr after the onset of mesenteric ischemia and the highest myoglobin levels were seen in samples taken six hr after mesenteric ischemia. Other findings were similar in both peripheral and mesenteric ischemia experimental groups.

Conclusion

We could mention that troponin T and myoglobin are not specific markers for mesenteric or peripheral ischemia. Therefore, there is no diagnostic value when they are in normal ranges. However, in patients with positive troponin or myoglobin without an underlying cardiac cause, it is likely that ischemia is present in another tissue.

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Declaration of interest

The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

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