Ischemia-Modified Albumin as an Early Diagnostic Marker for Acute Renal Ischemia and Infarction

Akut Renal İskemi ve İnfarktüs İçin Erken Bir Tanısal Belirteç Olarak İskemiyle Değişmiş Albumin

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ABSTRACT Objective: Acute renal infarction is a condition seldom encountered in emergency departments and it is generally diagnosed by chance when considering other diagnoses. This study investigates whether or not serum ischemia-modified albumin (IMA) levels rise in rats subjected to renal ischemia and infarction produced by clamping of the renal artery. **Material and Methods:** In this randomized, controlled and non-blinded trial, 24 mature male Wistar rats were divided into four, as two control groups (Groups I and III) and two intervention groups (Groups II and IV). In the control groups, blood was sampled in Group I at the 30th minute and in Group III at the sixth hour, following a simple laparotomy. In intervention groups II and IV, the renal artery was ligated leading to ischemia, and blood samples were taken at the 30th minute and sixth hour, respectively. **Results:** No difference was determined in terms of IMA levels in blood samples taken from the control and ischemia groups at the 30th minute (p= 0.12), although plasma IMA levels in the ischemia groups were significantly higher compared to those of the control groups in the 6th hour blood samples (p= 0.01). In addition, in the ischemia group, sixth hour blood sample levels were higher than the 30th minute sample levels (p= 0.011). **Conclusion:** Our preliminary findings suggest that there is no significant rise in IMA levels in the hyperacute period (first 30 minutes) of acute renal ischemia, however IMA levels measured at the sixth hour may represent a significant diagnostic parameter altough further studies are necessary.

Key Words: Ischemia; infarction; serum albumin

ÖZET Amaç: Akut renal infarktüs acil servislerde nadir rastlanan bir durumdur ve genellikle diğer tanılar üzerinde düşünülürken şans eseri tanı koyulur. Bu çalışma renal arterin klemplenmesi yoluyla renal iskemi ve infarktüse maruz bırakılmış sıçanlarda serumdaki iskemiyle değişmiş albumin (İDA) düzeylerinin yükselip yükselmediğini araştırmaktadır. **Gereç ve Yöntemler:** Bu randomize, kontrollü, kör olmayan çalışmada 24 ergin erkek Wistar sıçanı iki kontrol (Grup I ve III) ve iki girişim (Grup II ve IV) grubu olmak üzere dört gruba ayrıldı. Kontrol gruplarından Grup I'de basit laparatomiyi takiben 30. dakikada, Grup III'de ise altıncı saatte kan örnekleri alındı. Girişim grupları olan II ve IV'te renal arter iskemiye yol açacak şekilde bağlandı ve sırasıyla 30. dakika ve altıncı saatlerde kan örnekleri alındı. **Bulgular:** Altıncı saatte alınan kan örneklerinde iskemi gruplarında plazma İDA düzeyleri kontrol gruplarıyla karşılaştırıldığında belirgin olarak yüksek olduğu halde (p= 0.01) 30. dakikada iskemi ve kontrol gruplarından alınan örneklerde İDA düzeyleri bakımından hiçbir fark saptanmadı (p= 0.12). Ayrıca iskemi grubunda altıncı saatte alınan kan örneklerindeki düzeyler 30. dakikada alınanlara göre daha yüksekti (p= 0.011). **Sonuç:** İlk bulgularımız akut renal iskeminin hiperakut evresinde (ilk 30 dakika) İDA düzeylerinde önemli bir yükselme olmadığını düşündürmektedir fakat altıncı saatte ölçülen İDA düzeyleri önemli bir tanısal parametre olabilir ve bu nedenle daha ileri çalışmaların yapılması gereklidir.

Anahtar Kelimeler: İskemi; infarktüs; albumin

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Patients with acute renal infarction generally present to hospital emergency departments with stomach pain, pain in the lower abdominal quadrants or back pain. Such patients represent 0.007% of all applications.[1] Since renal infarction is seldomly seen, it is frequently confused with more frequenly observed stomach pain. Research on the subject has shown that diagnosis of renal infarction is generally achieved by chance while the patient is being treated for some other condition.[2] As the presentation of renal infarction to emergency departments imitates other disoders, it is important to keep renal infarction in mind in such circumstances.

Angiography, renal scintigraphy, intravenous pyelography and tomography are the techniques used in diagnosis.[1,3] Since these techniques require extra personnel and equipment, emergency departments need blood tests for early and practical diagnosis. No specific findings apart from high LDH and hematuria are found in patients' biochemical values.[1]

Ischemia-modified albumin (IMA) is a new biochemical marker for ischemic conditions. An increasing number of studies have shown that IMA levels rise in various acute ischemic conditions, such as cerebral, myocardial, pulmonary and mesenteric infarction, so that IMA can be used as a diagnostic marker.[4]

OBJECTIVES OF THIS INVESTIGATION

Our aim in this study was to investigate serum IMA levels on an experimental model of rats performed by renal arterial occlusion. In addition, we investigated whether IMA levels incrased in time or not. We also compared IMA levels with the other common ischemia markers, as lactate and malondialdehyde (MDA) levels.

MATERIAL AND METHODS

STUDY DESIGN

This was a randomized, controlled, non-blinded interventional animal study. The study protocol was approved by the Karadeniz Technical University Faculty of Medicine Animal Care and Ethics Committee.

SETTING AND SELECTION OF PARTICIPANTS

Twenty-four mature male Wistar rats weighing 240 g to 250 g were used. The animals were kept in steel cages at a room temperature of 22°C and were given water and standard rat chow until the day of the study. For the last 12 hours before the study they were given only water.

STUDY PROTOCOL

The study was performed on 24 male rats randomized and placed in each group as six rats. Intramuscular injection of 50 mg/kg of xylazine was used for general anesthesia. Breathing rate, pulse, sO_2, and body temperature were continuously monitored. A heating pad was applied during anaesthesia to maintain body temperature.

The first group (group I) underwent a simple laparotomy, blood samples being taken 30 min thereafter. The renal artery was ligated in the rats in the second group (group II) and blood samples were again obtained at the 30th minute. The third group (group III) underwent a simple laparotomy, blood samples being taken six hours thereafter. The renal artery was ligated in the rats in the fourth group (group IV), and blood samples were taken at the sixth hour. Four-centimetre-long abdominal midline incisions were made for laparotomy, and incisions were then sutured with 3-0 silk. The same incision was also used for the ischemia groups (groups II and IV), the renal artery was ligated with 3-0 silk at the aortic bifurcation. Blood specimens were extracted with relaparotomy. The samples for histopathological examination were taken after 30 minutes in groups I and II, and after six hours in groups III and IV.

LABORATORY ANALYSIS

IMA levels were measured. Plasma MDA and lactate levels were also measured at the same time for comparison with IMA values and the biochemist was blinded to the groups.

IMA measurement

Three millilitres of aortic blood samples were taken from each rat. After being placed in plain tubes containing separation gels, the samples were allo-

TABLE 1: Time dependent changes of IMA, lactate and MDA.

Groups	1		2		3		4	
	30th minute control		30th minute ischemia		6th hour control		6th hour ischemia	
Parameters	Mean (Min-Max)	25 - 75 Percentiles	Mean (Min-Max)	25 - 75 Percentiles	Mean (Min-Max)	25 - 75 Percentile	Mean (Min-Max)	25 - 75 Percentiles
IMA (ABSU)	0.23 (0.19-0.28)	0.21 - 0.25	0.29 (0.23-0.31)	0.26 - 0.31	0.28 (0.24-0.33)	0.25 - 0.32	0.37 (0.29-0.40)	0.33 - 0.38
Lactate (mg/dL)	20.0 (13.0-31.0)	17.0 - 24.0	26.0 (18.0-33.0)	18.0 - 33.0	19.5 (11.0-35.0)	11.0 - 30.0	16.5 (10.0-23.0)	12.0 - 20.0
MDA (nmol/mL)	428.2 (380.0-501.5)	405.6 - 460.9	499.7 (416.7-593.1)	464.9 - 555.1	436.4 (351.5-550.4)	356.1 – 532.7	550.6 (532.2-573.5)	535.1 - 557.0

IMA: Ischemia modified albumin. MDA: Malondialdehyde. ABSU: Absorbance Units

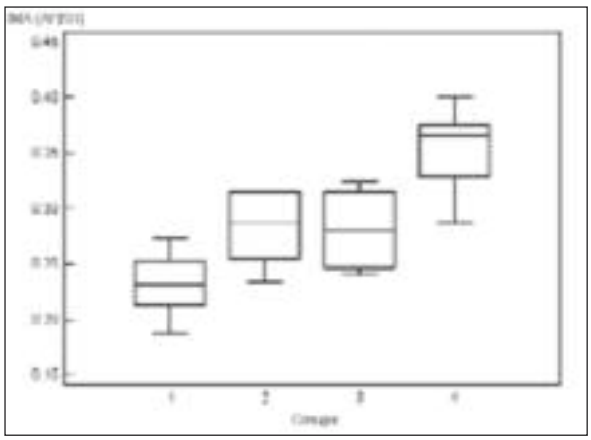

FIGURE 1A: IMA values of control and ischemia groups.
IMA: Ischemia modified albumin. ABSU: Absorbance Units

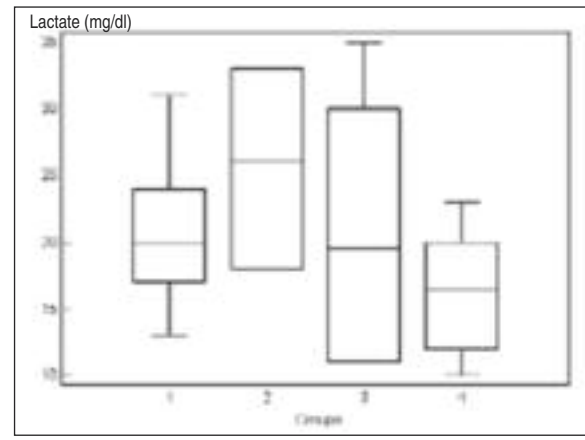

FIGURE 1B: Lactate values of control and ischemia groups.

The mean microscopic lesion scores were 0.33 ± 0.51 for group I, 0.50 ± 0.54 for group II, 0.66 ± 0.51 for group III and 3.5 ± 0.54 for group IV.

In the statistical analysis of the groups' histopathological scores; p values for the comparison of time dependent histopathological scores in the control groups (groups I and III) and ischemia groups (groups II and IV) were 0.57 for the 30th minute and 0.003 for the sixth hour; p value in the comparison of time dependent IMA levels in the control groups (groups I and III) was 0.26, and the p value in the comparison of time dependent levels in the ischemia groups (groups II and IV) was 0.003.

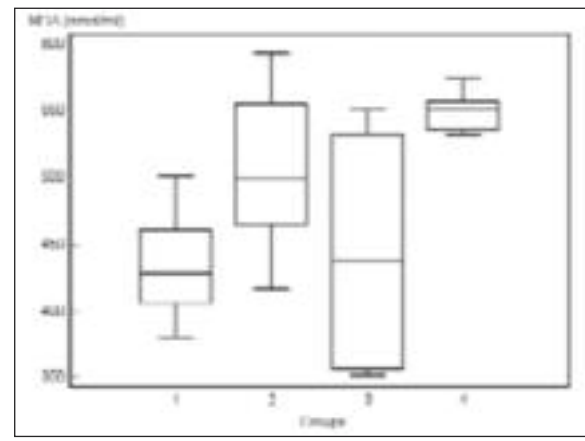

FIGURE 1C: Malondialdehyde (MDA) values of control and ischemia groups.

DISCUSSION

Many predisposing factors have been reported in acute renal infarction. Among these, atrial fibrillation, embolic events and ischemic and valvular coronary disease have been listed as major predisposing causes.[8,9] Trauma-related acute unilateral or bilateral renal infarction has also been reported.[10]

wed to clot for 30 minutes and were centrifuged at 3,000 *g* for 10 min before separating out the serum. The samples were then immediately frozen and stored at -80 °C for IMA assay.

Reduced cobalt to albumin binding capacity (IMA level) was analyzed using the rapid and colorimetric method described by Bar-Or et al;[5] 200 mL of rat serum was placed into glass tubes and 50 mL of 0.1% (w/v) cobalt chloride ($CoCl_2.6H_2O$; Sigma) added. After gentle shaking, the solution was left for 10 minutes in order to ensure sufficient cobalt albumin binding; 50 mL of 1.5 mg/mL dithiothreitol (DTT; Sigma) was added as a colorizing agent and the reaction was halted 2 min later by adding 1.0 mL of 0.9% NaCl. A colorimetric control specimen was prepared for every clamped and control specimen. For the colorimetric control samples, 50 mL of distilled water was substituted for 50 mL of 1.5 mg/mL DTT. Specimen absorbencies were analyzed at 470 nm using a spectrophotometer (Shimadzu UV1601, Australia). The colour of the specimens containing DTT was compared with that of the colorimetric control tubes, and the results were expressed as absorbance units (ABSUs).[5]

MDA measurement

Malondialdehyde (MDA) levels in plasma samples were established using the TBARS (Thiobarbituric Acid Reactive Substance) method developed by Yagi in 1994.[6]

Lactate Measurement

Lactate measurement was performed using a Roche vitreous chemistry 950 autoanalyzer.

Histopathological Examination

Following blood sampling, the rat kidneys were macro- and microscopically evaluated by a histologist blinded to the groups. Specimens were embedded in paraffin blocks and the sections stained with hematoxylin-eosin. Histological changes were evaluated by quantitative measurement of tubulointerstitial injury, assessed by counting the number of necrotic and apoptotic cells, loss of tubular brush border, tubular dilatation, cast formation, and neutrophil infiltration. The scoring was 0= none; 1= <10%; 2=11% to 25%; 3= 26% to 45%; 4= 46% to 75%; and 5= >76%. The data are expressed as mean values ± SD.[7]

Statistical Analysis

Statistical analyses were performed by using SPSS 13.0.1 software package (licence no: 9069728) and MedCalc statistical software program. Analysis of the simultaneous IMA, MDA and lactate levels in the simultaneous control and ischemia groups was performed using the Mann Whitney U-test. Time dependent variations in the control and ischemia groups were analyzed using Kruskal Wallis analysis of variance (Mann Whitney U-test with corrected Bonferroni test). P values less than .05 were considered as statistically significant.

RESULTS

BIOCHEMICAL PARAMETERS

Although no significant differences were determined in serum IMA, plasma MDA and lactate levels between the ischemia and control groups at the 30th minute (Group I and Group II), serum IMA and plasma MDA levels were significantly higher in the ischemia group (Group IV) when compared to those of the control group (Group III) in the sixth hour blood samples (for IMA; 30th minute p= 0.12, and sixth hour p= 0.01; for MDA; 30th minute p= 0.27, and sixth hour p= 0.028; for lactate; 30th minute p= 1, and sixth hour p= 1). Average IMA, MDA and lactate levels over time determined for the control (groups I and III) and ischemia groups (groups II and IV) showed statistically significant internal increases in ischemia groups for only IMA levels (p= 0.011), as shown in Table 1 and Figures 1A-C.

HISTOPATHOLOGICAL EXAMINATION

Following blood sampling, kidneys were evaluated macroscopically. There was no colour change or pathology in the control group. The normal pinkish colour of the kidney had changed in group II with the renal artery ligated, and the blood-sampled at the 30th minute had a bluish tinge. The colour was dark blue to violet in the ligated group IV, from which blood samples were taken at the sixth hour.

In addition to surgical procedures such as valve replacement, kidney transplantation, tumor surgery or aneurysm surgery can result in renal infarction as well as embolic material blocking the renal artery accidentally during catheterization.[1,11] Both hereditary and acquired clotting disorders also create a predisposition to renal infarction.[1] Vessel anomalies such as fibromuscular dysplasia, Marfan syndrome and Ehlers-Danlos syndrome can also create renal infarction.[12] Similarly, aortic dissection and aneurysm can also result in renal infarction.[13] Polyarteritis nodosa, systemic lupus erythematosus, Behçet's disease, infective endocarditis, paradoxical embolism, Henoch-Schönlein purpura, Chagas disease, intravenous injection, nasal insufflations of cocaine and smoking marijuana have also been linked to renal infarction. Many malignancies may also be accompanied by renal infarction.[14]

Unless renal infarction comes in mind it is difficult to diagnose, in the emergency department because it exhibits no specific findings. A number of studies have mentioned the importance of hematuria and high LDH levels in the diagnosis of renal infarction.[1,8] Studies in the literature also report an increase in SGOT, alkaline phosphatase, polyarteritis nodosa, C-reactive protein, white blood cell levels and polyarteritis nodasa in renal infarction.[8] It is reported that under normothermic conditions, the human kidney can tolerate 60-90 minutes of total ischemia, but total ischemia more than three hours leads to irreparable kidney function damage.[15] Therefore, in terms of early diagnosis, biochemical parameters suggesting renal ischemia in such patients may be extremely important.

During acute ischemic conditions, albumin's metal binding capacity for transition metals, such as copper, nickel and cobalt is reduced, thus giving rise to a metabolic variant of the protein, commonly known as IMA. The precise IMA generating mechanism is still unclear, though it seems that reactive oxygen species produced during ischemia may generate highly reactive hydroxyl free radicals, resulting in site-specific modification to the N-terminus of the albumin moiety, especially at the N-Asp-Ala-His-Lys sequence.[16]

IMA is a nonspecific marker of tissue ischemia which was previously studied in patients with acute chest pain and shown to increase in patients with myocardial ischemia, either spontaneously or subsequent to percutaneous coronary intervention.[5]

Extracardiac oxidative stress may elevate IMA levels and therefore, limit the usefulness of elevated IMA in the detection of cardiac ischemia.[17] This oxidative stress may be in any part of the body due to various causes. Gunduz et al. determined elevated IMA levels in mesenteric ischemia, and Turedi at al. determined elevated IMA levels in pulmonary embolism.[18,19] IMA is also elevated in other ischemic diseases such as limb ischemia and ischemic cerebrovascular accidents.[20-23]

The number of studies investigating the correlation between IMA levels and kidney damage or renal insufficiency is limited. In one recent study, Cichota et al. revealed a correlation between IMA levels and creatinine levels in patients with chronic renal insufficiency, and also determined a significant increase in anemia level and IMA levels in patients with final stage renal insufficiency.[24] Montagnana et al studied pre-and post-hemodialysis cardiac biomarker levels, and determined a reduction in post-dialysis cardiac biomarkers and a significant rise in IMA levels.[25] In another study, Sharma et al reported that IMA level was an important marker in predetermining mortality in patients with end stage renal insufficiency.[26] No studies have examined IMA level was patients with chronic renal insufficiency together with IMA levels in acute kidney damage. In the light of this, in our experimental study the samples taken at the 30th minute showing the hyperacute period in rats where acute renal ischemia and related kidney damage was established by ligation of the renal artery, it was determined that no histopathological ischemia findings had yet formed and that there was no significant difference in IMA levels in this period. However, the samples taken at the sixth hour showed ischemia-related histopathological changes and the serum IMA levels in this period statistically significantly increased in comparison with those in normal rats. A

similar correlation was determined in tissue MDA levels, regarded as a marker of tissue ischemia and oxidative stress, but no difference was determined in lactate levels, another ischemia marker, in either 30th minute or sixth hour samples.

LIMITATIONS

There are some limitations of this study. First, IMA is a new biomarker influenced significantly by a wide array of physiological variables including exercise and hydration. We were not able to control all those variables that might possibly influence IMA levels. Second, we did not compare other biochemical markers with IMA, except for lactate and MDA, in the diagnosis of acute renal infarct.

We used a non-commercial IMA test, which may be less reproducible than the standard commercial assay.

The study also had some limitations in terms of the model employed. Our study is controlled, however may not mimic typical acute renal infarct cases seen in practice.

CONCLUSION

Our preliminary findings from this limited and experimentally designed study suggest that in the hyperacute stage of acute renal ischemia (first 30 minutes) there is no significant rise in IMA levels, although IMA levels measured at the end of the sixth hour may be an important parameter in diagnosis, and further studies are needed.

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