Ischemia Modified Albumin Sensitivity of Coronary Artery Bypass Operation

Koroner Arter Baypas Operasyonunda İskemi Modifiye Albumin Duyarlılığı

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ABSTRACT Objective: Our objective in this study was to compare ischemia modified albumin (IMA) sensitivity with troponin and creatine kinase isoenzyme MB (CK-MB) in myocardial ischemia formed as a result of ischemia-reperfusion in coronary by-pass surgery. **Material and Methods:** 30 patients applying to our clinic within 6 months, to whom elective coronary artery bypass grafting (CABG) will be applied with the diagnosis of coronary artery disease (CAD), have been examined. Mean age of our patients was found to be 63,8±8,3 year for males (26 patients) and 58,5±3,4 (4 patients). Blood samples were taken from venous jugular catheter after anesthesia induction, before (T1), in the end (T2), and 1 hour after (T3) cardiopulmonary bypass (CPB). CK-MB, IMA, albumin, and troponin I measurements were carried out. **Results:** The three biochemical ischemia markers showed an increase as a result of reperfusion damage. IMA values were measured as 0.595±0.051 absorbance values (ABSU), 0.639±0.049 ABSU, and 0.589±0.47 ABSU in groups respectively. The increase in IMA measurements before and after CPB was found to be meaningful with myocardial ischemia exposure (p=0,002). IMA measurements at the pump outlet and 1 hour later were found to be meaningful with myocardial reperfusion result (p=0,001). When compared to IMA measurements before and 1 hour after CPB, it was determined that it reached its baseline value after ischemic reperfusion (p=0,899). **Conclusion:** In this study, the ischemia modified albumin showed a rapid increase after CABG when compared to troponin and CK-MB. It promises hope for early diagnosis of the myocardial damage.

Key Words: Ischemia-modified albumin; ischemia; cardiovascular surgical procedures

ÖZET Amaç: Bu çalışmada koroner baypas operasyonunda iskemi-reperfüzyon sonucu oluşan miyokard iskemisinde, iskemi modifiye albumin'in (İMA) duyarlılığının troponin ve kreatin kinaz izoenzim MB (CK-MB) ile karşılaştırılmasını amaçladık. **Gereç ve Yöntemler:** Çalışmamızda, 6 ay içerisinde kliniğimize başvuran, koroner arter hastalığı tanısı ile elektif koroner arter baypas grefteleme (CABG) yapılacak 30 hasta incelendi. Hastalarımızın yaş ortalaması; erkek 63,8±8,3 (26 hasta), kadın 58,5±3,4 yıl (4 hasta) olarak bulundu. Kan örnekleri; anestezi indüksiyonu sonrası kardiyopulmoner baypas (CPB) öncesi (T1), CPB bitiminde (T2) ve CPB sonrası 1. saatte (T3) venöz juguler kateterden alındı. CK-MB, İMA, albumin, troponin I ölçümleri yapıldı. **Bulgular:** Her üç biyokimyasal iskemi belirteci de iskemi reperfüzyon hasarı sonucu artış gösterdi. İMA değerleri gruplarda sırasıyla 0,595±0,051 ABSU, 0,639±0,049 ABSU ve 0,589±0,47 absorbans değerleri (ABSU) olarak ölçülmüştür. CPB öncesi ve sonrasında İMA ölçümlerinde artış miyokard iskemi maruziyeti ile anlamlı saptandı (p=0,002). CPB çıkışı ve 1. saat sonraki İMA ölçümleri miyokard reperfüzyon sonucu anlamlı bulundu (p=0,001). CPB öncesi ve 1. saat sonrası İMA ölçümlerde karşılaştırıldığında iskemi reperfüzyon sonrası bazal değerine geldiği tespit edildi (p=0,899). **Sonuç:** Bu çalışmada, iskemi modifiye albümin CABG sonrasında troponin ve CK-MB ile karşılaştırıldığında ilk dakikalarda hızlı artış göstermiştir. Miyokard hasarının erken tanısında umut verici bulunmuştur.

Anahtar Kelimeler: İskemi-modifiye albumin; iskemi; kardiyovasküler cerrahi girişimler

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An increase in cardiac ischemia markers after coronary bypass surgery (CABG) shows the degree of myocardial damage formed as a result of ischemia-reperfusion. Myocardial necrosis forms undesired severe clinical cases. The fact that it is 5 times more than baseline values of biochemical markers is one of diagnostic criteria for myocardial ischemia associated with coronary bypass.[1] Increases up to 20 times of upper limit of creatine kinase isoenzyme MB (CK-MB) were found to be associated with poor prognosis.[2] Again, high levels of troponin increases were found to be associated with severe clinical results.[3] The pathophysiological events of ischemia, including hypoxia and free oxygen radicals, result in a conformational change of the N-terminus of albumin and this new molecule is called ischemia-modified albumin (IMA).[4] IMA was found to be high after percutaneous coronary intervention (PCI).[5,6] Again, it was found to be high after acute coronary syndrome.[7] It was found that it increased after CABG surgery.[8] In our study, our aim was to compare the IMA sensitivity in myocardial ischemia formed following the CABG surgery with troponin and CK-MB.

MATERIAL AND METHODS

30 elective isolated coronary bypass patients were included in the study between January-June 2009. Ethical Committee approval of Dokuz Eylül University and voluntary patient written informed consent were obtained. Cases with acute coronary syndrome and myocard infarction in the last 1 month were excluded from the study. Exclusion criteria were indicated in Table 1.

Patient demographical data were indicated in Table 2. Also, standard CABG surgery was applied together with surgical and anesthetic team. Distal anastomosis was completed with cold blood cardioplegia protection under hypothermic cardiac arrest and intervals of 20 minutes. Mean blood pressure was observed at the level of 50-70 mmHg. Hematocrit values of the patients during the CPB were maintained between 18-25%. All of the patients received an internal mammary artery to descending left anterior except for 1 redo-CABG. Intra-aortic balloon counterpulsation (IABP) was introduced in one case during weaning from CPB.

Blood samples were taken from venous jugular catheter after anesthesia induction, before (T1), in the end (T2), and 1 hour after (T3) cardiopulmonary bypass (CPB). CK-MB, IMA, albumin, and troponin I measurements were carried out. Plasma samples were taken into 8 cc gel separator tubes is used to measure IMA, albumin, troponin I and CK-

TABLE 1: The exclusion criterias.

Plasma albumin concentrations under 2 g/dL and over 5.5 g/dL,
Stroke,
Transient ischemic attack,
Peripheral vascular disease,
Muscle disease,
Trauma,
Shock,
Malnutrition,
Pregnancy,
Liver diseases,
Renal failure,
Neoplasies,
Acute coronary syndrome and myocard infarction in the last 1 month.

TABLE 2: The demographic characteristics of the patients.

Age (years)	63.1 ± 8.2
Male, n (%)	26(86.6)
Diabetes, n (%)	7 (23.3)
Hypertension, n (%)	22 (73.3)
Atrial Fibrillation, n (%)	5 (16,6)
Smokers, n (%)	9 (34,3)
Number of affected coronary arteries, n (%)	
2 vessel	4 (13.3)
3 vessel	17 (56.7)
4+ vessel	9 (30)
Cardiopulmonary support time (min)	94±28.9
Cross-clamping time (min)	51.4±13.92
Grafts/patient	3.16±0.6
IMA (internal mammary artery) graft use, n (%)	29 (96,6)

MB. Blood samples were centrifuged after 10 minutes and plasma examination was performed at -20°C. IMA levels were measured as being spectrophotometric with albumin cobalt binding test which is defined in the literature, troponin I and CK-MB measurements have been made with chemiluminescence, and plasma albumin levels with bromocresol purple method in auto-analyzer as spectrophotometric.[4-9] Albumin-cobalt binding test which is defined by Bar-Or et al. depends on colorimetrical measurement of colorful complex which is come out dithiothreitol (DTT) and cobalt which is not bound albumin and which is added the sample. For the measurement 0.1% of cobalt chloride solution, 0.9% NaCL solution (Eczacıbaşı-Baxter), glass tube, vortex, automatic ependorff pipet, single use plastic micro boxes and Shimadzu UV-1201V spectrophotometer are used. Cobalt chloride solution has been prepared by the distilled water (BOME) solution of the chemicals $CoCI_2.6H_2O$ (Sigma-Aldrich Lot:S38901-248 Kat: 20,218.5) DTT solution DTT (Sigma-Aldrich Lot: D5545-1G Kat: 117K0663). After addition of 50 ml 0.1% cobalt chloride solution into 200 ml patient plasma, the mixture was vortexed, and incubated for albumin-cobalt biding for 10 minutes. At the end of the incubation, 50 ml 1,5 mg/mL DTT solution is added into cobalt which does not bide to albumin in order to have the color reaction and is waited for 2 minutes. After that, the reaction is completed by adding 1 ml 0.9% NaCL into the mixture. The same steps have been made at the same time for the sample cure prepared with distilled water instead of DTT. In the end of the reactions, the differences of absorbance values (ABSU) read in 470 nm were recorded as IMA figures.[8,9]

In all calculations and statistical analyses, the program 'Statistical Package For Social Sciences' (SPSS-Chicago, IL, USA) 15 and Software Excel (Microsoft-USA) were used. The results were valued in 95% correctness as the average and standard deviation. The appropriateness to normal expedition has been checked. In order to understand whether the changeable values shows a normal expedition or not, Kolmogarov-Simirnov test has been made. Data analysis was based on non-parametric statistical methods due to the small sample and the abnormal distribution of the enzymes. Kruskal-Wallis test was applied for data that does not show the normal distribution. If the changeable values showed normal expedition, ANOVA test was used. Pearson correlation test was used for the evaluation of the times of cross-clamp and ischemia modified albumin levels. If that p-value was <0.05, the results were considered as significant.

RESULTS

The increase in IMA measurements before and after CPB was found to be statistically meaningful with myocardial ischemia exposure (p=0,002). IMA measurements at the pump outlet and 1 hour later were found to be meaningful with myocardial reperfusion result (p=0,001) and an increase was determined. When compared to IMA measurements before CPB and 1 hour after the termination of CPB, it was seen that the ischemia was reduced to its baseline value different for each patient after the reperfusion (p=0,899) (Figure 1). As a result of the test, it was found that there was a valuable difference in albumin levels between the groups (p:0.000). It was observed that albumin value was generally reduced after the CPB but was higher than CPB increase in 1 hour after the CPB. When IMA was compared with albumin levels, we saw that there was no correlation between them. CK-MB results were statistically significant, when we compared three groups with each other (p:0.000). An increase tendency was observed in the three samples (T1, T2, and T3) as a result of CK-MB, troponin I, and myocardial necrosis. As seen in the Figure-1, during T2 and T3 measurements, troponin 1 had more peaks than CK-MB. When we compared 3 groups with cross clamp time; we saw that there was a negative significant correlation with the group 2 IMA levels (r:-0,473, p:0,008). IMA concentrations were negatively correlated with the duration of cross clamping, where lower levels of IMA were detected in cases with prolonged cross clamping time. The reason of this re-

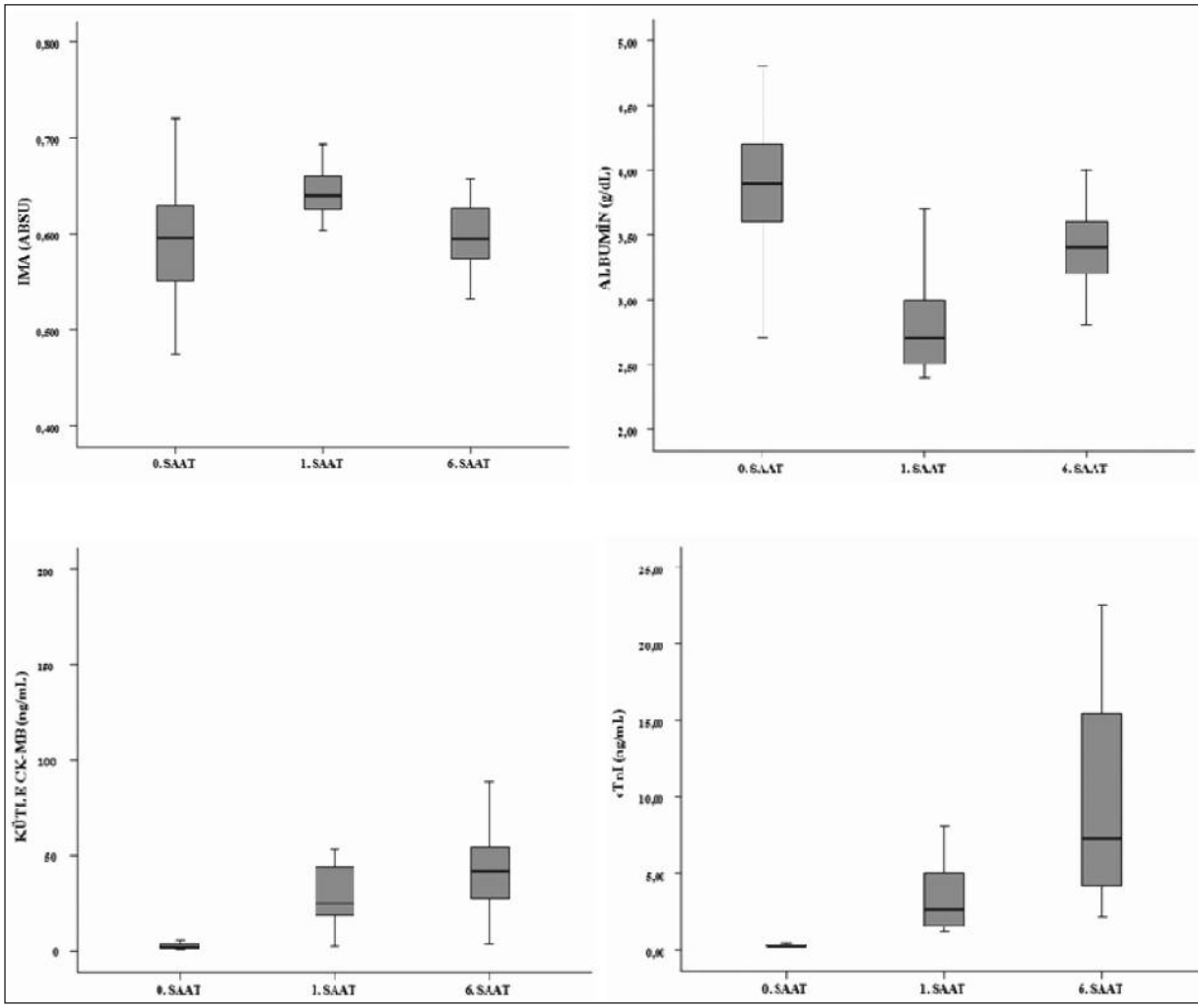

FIGURE 1: Evaluation of IMA, Albumin, CK-MB, and Troponin I levels.

sult is rapid increase of IMA levels during early stages of ischemia. There were no significant relations between T3 IMA samples and cross-clamp times (p:0,055, r:-0,355). When the cross-clamp times and CK-MB, troponin I levels were analyzed, it was seen that there was a positive correlation between the cross-clamp times and CK-MB, troponin (r:0,630- r:0,384) in T3 samples. The rising pattern of myocardial necrosis of the two enzymes was observed.

DISCUSSION

Initial value of IMA biochemical marker observed before CPB and the measurement after CPB in CABG surgery showed an increase. We observed that it showed a peak increase as early indicator of the ischemia developed as a result of CPB and returned to its baseline value in approximately 1 hour after CPB. Thus, it was realized that the IMA was increased meaningfully in the ischemia developed after CPB and returned to its baseline value in 1 hour (T3) after CPB depending on its coronary revascularization.

Recently, the last amino terminal in albumin structure is the region where transition metals such as cobalt, nickel, and copper are bound. Free metal binding capacity of the IMA is lower than that of normal albumin. It is known that N-terminal region of the albumin is binding zone of bivalent transition metals such as copper (Cu^{+2}), nickel

(Ni^{+2}), and cobalt (Co^{+2}).[10-12] Depending on the hypoxia formed due to the ischemia, it was especially determined that modification happened in N-terminal zone of the albumin as a result of an increase in reactive oxygen radicals, and its cobalt binding capacity was reduced as a result of this modification.[11,12] This modified albumin is called as ischemia modified albumin. IMA levels are measured by means of a colorimetric test defined by Bar-Or et al. and also approved by Food and Drug Administration (FDA).[12,13]

CPB has a destructive effect on all of the tissues and organs despite all technical developments and increasing experiences.[14] It may cause inadequate myocardial protection, reperfusion injury, incomplete target vessel revascularization, myocardial ischemia, and necrosis in CABG surgery. CPB causes complement system activation by systemic inflammatory response syndrome (SIRS). Thus, it affects the plasma proteins such as albumin by providing cellular stress and vascular permeability.[15] This condition is one of the reasons of low albumin levels in the blood sample taken after the CPB. In our study, IMA measurements gave results independent from albumin variations similarly to the literature.[8-16] High IMA results are also observed in myocardial ischemia as well as end-stage renal failure, muscle diseases, intestinal ischemia, cerebrovascular ischemia, pregnancy, pulmonary embolism, and peripheral vascular diseases.[16-19] Also, it is reported that the IMA can be the indicator of oxidative stress.[16,17] IMA increases as a result of reactive oxygen species productions formed during CPB.[20] In a study carried out as PCI temporary myocardial ischemia model, IMA leves were observed in serum samples obtained before, after, and 6 and 24 hours after PCI attempt. IMA levels observed at the end of 6 and 24 hours were found to be at initial levels while IMA levels increased.[21] Again similarly, it was determined that IMA levels increased immediately after the attempt and in 30 minutes in blood samples taken before, after, 30 minutes after PCI attempt and at 12 hours and returned to its baseline values.[22] IMA level increases within minutes after myocardial injury and tends to be elevated for 6 hours, even the perfusion has been restored.[21,22]

TABLE 3: Evaluations of the patients.

	Albumin (g/dL)	Troponin (ng/mL)	CK-MB (ng/mL)	IMA (ABSU)
T1 (n:30)	3.82±0.55	0,28±0,15	2,84±2.55	0,595±0,051
T2 (n:30)	2,78±0,32	3,47±2,19	30,87±19,72	0,639±0,049
T3 (n:30)	3,39±0,34	9,97±6,49	47,10±33,36	0,589±0.47
p values				
T1-T3	0	0	0	0,899
T1-T2	0	0	0	0,002
T2-T3	0	0	0	0,001

IMA levels increased just after the CABG. We observed a significant increase between all measurements of CK-MB and troponin I biomarkers. As seen from Figure 1, Table 3 during and after CPB measurements, the troponin I has more peaks than CK-MB which has less sensitivity and specifity than troponins.[22] Thus, it is understood that troponins are better biochemical markers than CK-MB in defining little myocardial injury as stated in the literature.[21,22] IMA is a good marker for early ischemia.[7-22] Progression of ischemia to infarction during prolonged cross clamping may be the result of decreased IMA levels. Significant increase in necrotic markers, troponin, and CK-MB has supported this finding in our study. It was seen that there was a positive correlation between the cross-clamp times and CK-MB and troponin (r:0,630-r:0,384). Cellular stress factors, free oxygen radicals, and high levels of lactic acid are other additional factors affecting the IMA levels.[19-22] IMA is an indicator of a reversible ischemia.[7-22,23] IMA levels tend to decrease after 1 hour of CPB with reperfusion of the myocardium. These results showed that IMA levels can be a good marker for detecting the myocardial injury earlier.[23,24] IMA levels become normalized to baseline levels before CK-MB and troponin.

Obtainment of biochemical parameters for the ischemia likely to occur perioperatively after CPB or in intensive care unit (ICU), and immediately interference in minutes (inotropes or coronary dilatators support, intra-aortic balloon pump, pace-

maker support etc.) can be life-saving for the patients. Besides, detection of myocardial injury, recognition of the patients earlier, and noticing the risk allow the increase in the quality and cost-effectiveness of patient-care.

The effect of recent developments in the technology, the existence of minus 1 g myocardial necrosis can be defined through sensitive biochemical markers.[25] Probably in a very near future, a multi-marker strategy including myoglobin, IMA, CK-MB, troponins with some combinations of inflammatory markers like C-reactive protein (CRP) and cardiac biomarkers in early diagnosis and treatment protocols of myocardial ischemia will be accepted. Among all these studies, the marker mostly gaining importance is ischemia modified albumin, and approved by FDA. The consequences are financially beneficial. Successful results and healing in patient prognosis will increase the need for such kind of innovative protocols. IMA levels increase just after CABG operations. However, daily routines have not been suggested in long-term follow up for measuring them nowadays.

CONCLUSION

Early diagnose and treatment in coronary ischemia is a life-saving condition. Markers still used in clinical practice are inadequate for detecting the myocardial ischemia without necrosis. IMA holds promise as an exciting modality because of its ability to detect myocardial ischemia before the onset of necrosis.

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