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SERUM LEVEL OF ISCHEMIA MODIFIED ALBUMIN AND TOTAL OXIDATIVE STRESS AS A BIOMARKER FOR DIAGNOSIS OF ISCHEMIC HEART DISEASE PRONE TO MYOCARDIAL INFARCTION

Uzma Riaz[1*], Najma Majeed[2], Mehwish Iftikhar[3]

[1]Associate Professor Pharmacology, Benazir Bhutto Medical College, Mirpur, AJK
[2]Assistant Professor Biochemistry, Mohtarma Benazir Bhutto Shaheed Medical College, Mirpur, AJK
[3]Assistant Professor Biochemistry, King Edward Medical University, Lahore, Pakistan

***Corresponding Author:** Uzma Riaz
*Associate Professor Pharmacology, Benazir Bhutto Medical College, Mirpur, AJK
Email: Uzmariaz2@gmail.com

Abstract

Background: Albumin is the most abundant protein in human blood plasma and its level may vary according to age. In neonates, its level is approximately 3.9 g/dl. It decreases to 2.8 g/dl at 9 months of age and increases slowly (3.5 g/dl to 5.5 g/dl) until the adult age. For albumin perform its main functions - to maintain the colloid osmotic pressure of intravascular fluid and to bind several substances in blood plasma such as bilirubin, fatty acid, calcium ion, magnesium ion and various drugs.

Objective: To determine the Serum level of Ischemia Modified Albumin and total oxidative stress as a biomarker for diagnosis of ischemic heart disease prone to myocardial infarction.

Methodology: This retrospective study was done at the Department of Biochemistry Mohtarma Benazir Bhutto Shaheed medical college Mirpur. The study duration was one year from March 2023 to March 2024. A total of 120 patients were enrolled in our study. Patients were divided into case group and control group. Case group was further categorized as group A, group B and group C. In group A, subjects with diagnosed Ischemic Heart Disease (IHD), in group B, subjects performed PCI and in group C, subjects with diagnosed MI were included. In control group, normal, healthy subjects with same age as matched to case groups were selected for comparison. IMA assayed by using colorimetric method using dithiothreitol (DTT). Troponin-I was determined by using ELISA kit method. Superoxide Dismutase (SOD) and total antioxidant capacity (TAC) were determined by spectrophotometer. To determine triglycerides (TAG), GOD- PAP method on selectra Pro Sa fully automated analyzer was used. Serum total cholesterol was analyzed by Chod-Pap method on selectra Pro Sa fully automated analyzer. Homogeneous enzymatic colorimetric method was used for estimation of high-density lipoprotein (HDL)– cholesterol and direct method was used for low density lipoprotein (LDL)-cholesterol measurement. All the controls were also subjected to measurement of above biochemical parameters along with estimation of serum IMA. Data was analyzed using Statistical Package for Social Sciences (SPSS), version 24.0.

Results: A total of 120 patients were enrolled in our study with 60 in control group and 60 in case group. Statistically significant results were observed for total cholesterol, HDL and LDL (p=<.05),

while results for TAG were insignificant (p=>0.05). High mean (± SD) values of IMA (u/ml) were observed for group C (96.11 ± 11.09) followed by group B (88 ± 4.99) and group A (83.11 ± 9.66) as compare to control group (23.99 ± 8.84). Statistically significant results were observed for IMA, troponin-I, SOD and TAC between group A, B, C and control group.

Key words: Serum; Ischemia Modified Albumin; Total oxidative stress; Myocardial infarction

Introduction

Albumin is the most abundant protein in human blood plasma and its level may vary according to age. In neonates, its level is approximately 3.9 g/dl. It decreases to 2.8 g/dl at 9 months of age and increases slowly (3.5 g/dl to 5.5 g/dl) until the adult age[(1)]. For albumin perform its main functions - to maintain the colloid osmotic pressure of intravascular fluid and to bind several substances in blood plasma such as bilirubin, fatty acid, calcium ion, magnesium ion and various drugs[(2)] - these levels must be maintained. Albumin contains 585 amino acids and, under normal conditions, the N-terminal region of this protein forms the N-Asp-Ala-His-Lys sequence. The first three amino acids show greater metal-binding capacity and specificity. Although this region contains an inherent affinity site for cobalt (Co), it also binds tightly to copper (Cu) and nickel (Ni)[(1-4)]. However, when exposed to ischemia, hypoxia and/or free radical damage, the N-terminal region of albumin is more susceptible to degradation when its ability to bind to metals is reduced, forming ischemia-modified albumin (IMA)[(2,3)].

The reduction of albumin affinity by Co, Ni and Cu, caused by the change that occurs at the N-terminal region, increases the concentration of these free metals in the blood[(3)]. Such change can occur within minutes after an ischemic event[(5)] and quickly elevates the IMA levels in the blood[(1)]. Therefore, some studies proposed the use of IMA as a useful rule-out marker for the diagnosis of acute coronary syndrome[(1,5,6)]. The term acute coronary syndrome (ACS) refers to any group of clinical symptoms compatible with acute myocardial ischemia, including angina and myocardial infarction (MI). This ischemic process is a result of insufficient blood flow in cells and inadequate oxygen and nutrient supplies to the site affected. According to the World Health Organization (WHO), the diagnosis of ACS may be based on three criteria: clinical symptoms, alterations in the electrocardiogram (ECG), and biochemical markers. However, these criteria have low specificity and sensitivity, indicating that the clinical symptoms are not specific enough, although their report is necessary; ECG shows 50% sensitivity; and, finally, the biochemical markers frequently used present late results, after tissue injury[(1)].

Currently, the biochemical diagnosis of ACS is accomplished by the myocardial necrosis biomarkers most commonly used: cardiac troponins, creatine kinase-MB fraction (CK-MB) and total creatine kinase (total CK). However, these biomarkers increase after tissue injury, approximately 4 to 6 hours after the cardiac event[(5)] and detect only the consequences of prolonged ischemia. CK (EC-2.7.3.2) has several functions in cellular energy metabolism. It catalyzes the reversible transfer of the phosphoryl group from phosphocreatine to adenosine diphosphate (ADP), to regenerate adenosine triphosphate (ATP)[(7)]. The major CK isoenzymes, whose names are given as a reference to the tissues in which they were historically isolated, creatine kinase BB fraction (CK-BB), and creatine kinase MM fraction (CK-MM) are found in the cytosol. Both isoenzymes exist as homodimers under specific physiological conditions and may be present as a heterodimer CK-MB in the heart. CK-MM and CK-MB isoforms can be easily detected in human serum. CK-MM is the main isoenzyme found in striated muscle (approximately 97% of the total CK). CK-MB is mainly found in cardiac muscle, where it comprises 15% to 40% of the total CK activity. However, trace amounts of CK-MB are found in skeletal muscle, therefore, patients with skeletal muscle injury will have increases in the absolute concentrations of total CK and CK-MB, but not associated with myocardial injuries. For this reason, it is used in combination with total CK and CK-MB measurements, and with cardiac troponins for the

diagnosis of ACS. The regulatory troponin complex plays an important role in the regulation of striated muscle contraction. It consists of three different subunits - troponin C (TnC), troponin I (TnI) and troponin T (TnT). TnT and intracellular TnT are mostly bound to myofibrils in the cardiac myocyte, although a small amount of TnT (6%-8%) and TnI (3% to 4%) is found in the cytoplasm of myocardial cells. The elevation of both troponins in plasma is due to the continuous loss of myofibrils caused by ischemia. The great amount of troponins in myocytes suggests higher sensitivity and specificity of this test when compared to other markers[8,9]. Data and clinical studies have shown that TnI is an early marker of myocardial injury[10]. However, it is important to recognize that troponin elevations can also be detected in conditions other than ACS, for example, in pulmonary embolism, stroke and severe renal insufficiency[8,9].

Although the biomarkers most widely used for the diagnosis of ACS are cardiac troponins, we must consider that improving the diagnosis of myocardial ischemia is still required, since it occurs before tissue necrosis, i.e. before MI. Despite the fact that ECG (along with the stress tesingt) is the most commonly used, it is not considered the gold standard for diagnosing heart diseases[11].

IMA has been recently licensed by the US Food and Drug Administration (FDA) for the diagnosis of suspected myocardial ischemia. It is considered a very sensitive marker of myocardial ischemia. Although it has a high negative predictive value, IMA detection can corroborate the early diagnosis of cardiac ischemia and other existing conventional biomarkers[12]. However, high levels of IMA are found in many inflammatory diseases and also in diseases associated with oxidative stress, but little is known about the IMA levels in patients with ACS.

Considering that the N-terminal region of albumin is modified when exposed to ischemia, hypoxia, acidosis and free radical damage, and that its presence in the serum is an indicator of abnormalities, the main objective of the present study was to evaluate Serum level of Ischemia Modified Albumin (IMA) and total oxidative stress as a biomarker for diagnosis of ischemic heart disease prone to myocardial infarction.

Materials and methods

This retrospective study was done at the Department of Biochemistry Mohtarma Benazir Bhutto Shaheed medical college Mirpur. The study duration was one year from March 2023 to March 2024. Study approval was taken from the Ethics committee of the hospital. The inclusion criteria for our study were all the patients who developed myocardial ischemia admitted for monitoring, patients with percutaneous coronary intervention (PCI) and patients diagnosed as myocardial infarction (MI) with increased cardiac troponin-I (cTnI) level whereas the Known cases of liver disease, renal disease, peripheral vascular disease, brain ischemia, taking any lipid lowering drugs and women with pregnancy, were excluded from the study. A total of 120 patients were enrolled in our study. Patients were divided into case group and control group. Case group was further categorized as group A, group B and group C. In group A, subjects with diagnosed Ischemic Heart Disease (IHD), in group B, subjects performed PCI and in group C, subjects with diagnosed MI were included. In control group, normal, healthy subjects with same age as matched to case groups were selected for comparison. About 8 milliliters of blood was drawn from the antecubital vein from each subject, collected in clean centrifuge tube. The blood sample centrifuged for 5–10 minutes at 2500 – 3000rpm. The serum stored in clean, plastic dry cups at -80 degrees. IMA assayed by using colorimetric method using dithiothreitol (DTT). Troponin-I was determined by using ELISA kit method. Superoxide Dismutase (SOD) and total antioxidant capacity (TAC) were determined by spectrophotometer. To determine triglycerides (TAG), GOD- PAP method on selectra Pro Sa fully automated analyzer was used. Serum total cholesterol was analyzed by Chod-Pap method on selectra Pro Sa fully automated analyzer. Homogeneous enzymatic colorimetric method was used for estimation of high-density lipoprotein (HDL)– cholesterol and direct method was used for low density lipoprotein (LDL)-cholesterol

measurement. All the controls were also subjected to measurement of above biochemical parameters along with estimation of serum IMA (Biswas et al., 2014) Height was calculated to the nearest of 0.1cm while patients standing straight, and weight was calculated with a moveable weighing machine with zero error of 0.1 kilogram (kg). Body mass index (BMI) was measured as the ratio of weight (kg) to height squared (m2). Data was analyzed using Statistical Package for Social Sciences (SPSS), version 24.0. Continuous variables were presented as Mean ± SD. One-way ANOVA was employed for comparative analysis of groups. P value <0.05 was considered as statistically significant.

Results

A total of 120 patients were enrolled in our study with 60 in control group and 60 in case group. Of case group, 20 subjects each were included in group A, B and C. Figure 1 shows the comparison of baseline and biochemical parameters between control group with different study groups. The mean age (±SD) in control group was 45.99 (±4.22) years, in group-A was 48 (±4.48) years, in group-B was 48.02 (±4.88) years and in group-C was 50.99 (±5.81) years. Mean BMI (SD) in control group and group A, B, and C were found to be 23 (± 1.99) kg/m2, 23.11 (± 2.11) kg/m2, 23.31 (± 1.21) kg/m2 and 24.12 (± 1.88) kg/m2, respectively. Statistically significant results were observed for total cholesterol, HDL and LDL (p=<.05), while results for TAG were insignificant (p=>0.05). Comparison of IMA, Troponin-I, SOD and TAC in control group with case groups were shown in Figure 2. High mean (± SD) values of IMA (u/ml) were observed for group C (96.11 ± 11.09) followed by group B (88 ± 4.99) and group A (83.11 ± 9.66) as compare to control group (23.99 ± 8.84). Mean ± SD of troponin-I, SOD and TAC values were 0.13 ± 0.04, 3.14 ± 0.021 and 1.49 ± 0.041 in control group, 0.15 ± 0.07, 2.44 ± 0.05 and 0.99 ± 0.04 in group A, 0.20 ± 0.044, 2.55 ± 0.071 and 1.04 ± 0.047 in group B and 0.66 ± 0.089, 2.36 ± 0.083 and 1.09 ± 0.031 in group C, respectively. Statistically significant results were observed for IMA, troponin-I, SOD and TAC between group A, B, C and control group.

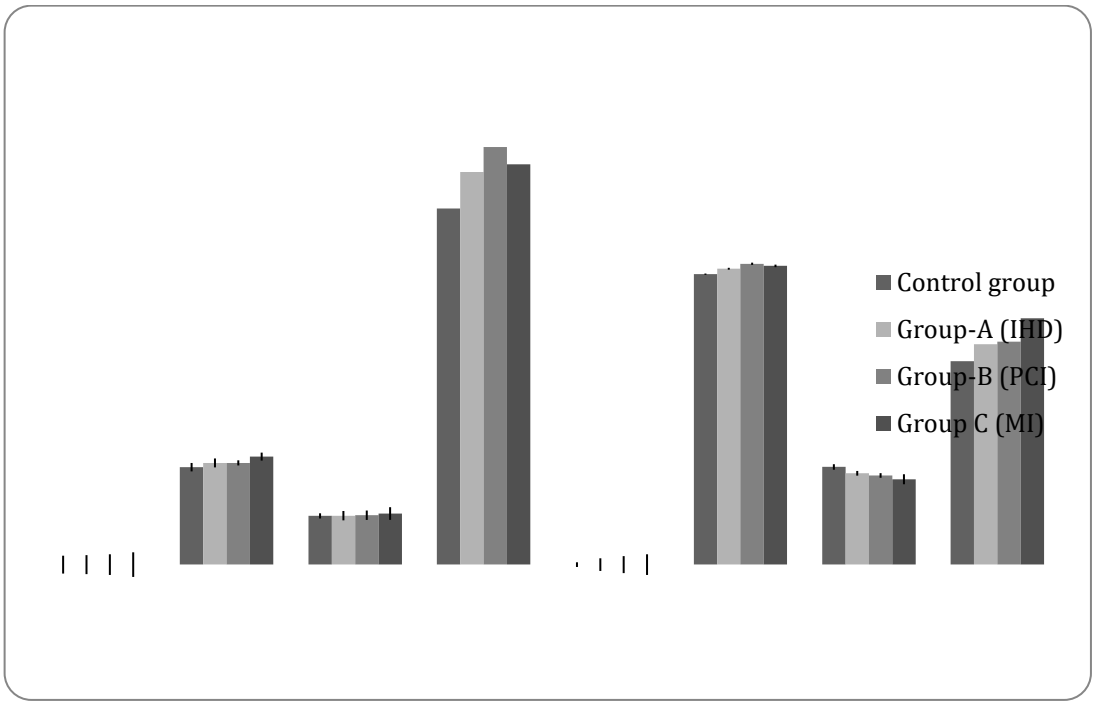

Figure 1: Comparison of baseline and biochemical parameters between control and different study groups.

Figure 2: Comparison of IMA, Troponin-I, SOD and TAC in control and case groups.Discussion

MI is now considered part of a spectrum referred to as acute coronary syndrome and is one of the main events caused by myocardial ischemia that can result in irreversible myocardial cell damage or death[16]. Some studies suggest that unlike injury and cellular necrosis markers, such as total CK, CK-MB and TnI, IMA can be used as a marker for the early prediction of myocardial ischemia[1,15,16]. According to Sinha[18], IMA sensitivity for the diagnosis of acute ischemic chest pain is significantly higher than that of ECG and TnT. These results corroborate the findings of Christenson[5], who also observed high sensitivity and high negative predictive values of IMA, demonstrating that the ACB test could be used to safely identify low-risk patients, and therefore, reduce the admission of patients in emergency hospitals. However the presence of IMA may not confirm myocardial ischemia but other medical conditions such as diabetes *mellitus*, peripheral vascular disease, glaucoma, skeletal muscle ischemia and systemic sclerosis[12].

In our study, significant elevated levels of IMA in group C followed by group B and A were observed as compare to control group. Our results are comparable with Sahin et al., who reported that IMA levels amongst patients assessed in the emergency department by pre-diagnosis of ACS was significantly greater than values in healthy control group.[17] Levels of IMA were also found to be in chest pain high in another study.[18] Few studies also recommended that instead of injury and cellular necrosis markers, such as total CK, CK-MB and Tn-I, IMA is a marker for the early prediction of myocardial ischemia[19] However, total CK, CK-MB were not determined in our present study. Nevertheless, IMA displayed no significant difference and correlation between IMA and the cardiac markers in Bonorino et al., (2015) study. It was found that IMA cannot be used unaccompaniedfor the identification of MI because outcome may hinge on the concentration of serum albumin, which could not be detected in our patients [20]. In addition, Reddy et al. (2014) demonstrated that IMA can be an early predictor of Tn-I results after 6-24 hours in patients with ACS, suggesting an association between IMA and Tn-I. To the best of our knowledge, this is the first report of its kind from this region of world highlighting that the novel biomarker has several possible utilities including the diagnosis of many conditions, differentiating IHD from non-ischemic and even prognostic value. Increased levels of IMA evidently forecasted adverse results in patients and increased the hospitalization days[21]. The TG within all Apo lipoprotein B and most HDL particles were associated with higher risk of MI. In present study, TG was non-significantly increased in group B followed by group C and A. While, significantly low HDL was observed for case groups (mainly in group C) as

compared to control group. To decrease risk for MI and cardiovascular events, focusing on targeting treatment for low serum levels of HDL was required. In this study, significant results were observed for troponin-I, SOD, TAC and IMA as a biomarker for the diagnosis of MI, IHD and PCI. Higher IMA level by Guntas et al. 2017 is a marker of oxidative stress in diseases with inflammation. When reperfusion of MI occurs, recognized as an alternative to AMI develop an inflammatory reaction in tissues[22]. However, the restoration of blood flow in ischemic tissue extends the related tissue damage to ischemia. Present study used SOD and TAC as safeguard to MI, IHD and PCI to defend the oxidative stress. Hence, IMA seems to be a valuable marker to be used in patients at initial or late stages of ACS and coming to emergency department[23]. (However, further clinical trials with larger number of patients are required to address the utility, outcomes, and cost-effectiveness of IMA prior to its integration into clinical practice [24].

Conclusion

This study concludes that serum IMA at emergency department admission facilitates the early diagnosis of IHD. It should be regularly estimated in combination with other cardiac biomarkers.

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