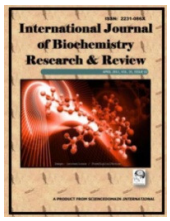

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Arginase Activity as a Useful Cardiac Marker for Differential Diagnosis of UA and MI: A Pilot Study

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAA designed the study, wrote the protocol and supervised the work. Authors CGY and PNJ carried out all laboratories work and performed the statistical analysis. Authors CGY and PNJ wrote the first draft of the manuscript and the literature searches. Author SAA edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study was undertaken to find the levels of aspartate transaminases (AST), creatine kinase (CK-MB) and arginase in serum sample of patients under study and check effectiveness of arginase as a marker in acute coronary syndrome (ACS), and to find the correlation between AST and CK-MB with arginase in unstable angina (UA) and myocardial infarction (MI).
Study Design: Case control study carried out at department of Biochemistry, B. J. Medical College Pune, Maharashtra, India, between Sept 2011-Dec 2013.
Methodology: The study comprised of clinically diagnosed 60 patients: 30 of UA and 30 of MI. Age and sex matched 30 patients were studied as controls. Blood sample were collected from individual under study, serum was separated and used to estimate levels of CK-MB, AST and arginase.
Results: The levels of AST, CK-MB and arginase were estimated and the results were compared with that of control. The levels of arginase were elevated significantly (P<0.001) like AST and CK-

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MB in patients with MI as compared to UA. In the study there was no correlation observed between CK-MB and arginase in patients of UA or controls. The same was true with the levels of AST and arginase. On the other hand a strong positive correlation was found between CK-MB and AST with arginase in MI patients.

Conclusion: It can be concluded that like AST and CK-MB, arginase also increases with progression of ACS. Thus arginase can be used as sensitive marker to differentiate between UA and MI.

Keywords: Arginase; coronary artery disease; myocardial infarction.

1. INTRODUCTION

Coronary artery disease (CAD) is the leading cause of mortality and morbidity in the world and acute coronary syndrome (ACS) is the commonest amongst it. The prevalence of coronary artery disease (CAD) in India is 6.4% in urban and 2.5% in rural area. India has the highest burden of ACS. ACS is classified as non-ST elevation myocardial infarction (NSTEMI), ST elevation myocardial infarction (STEMI) and unstable angina (UA) [1]. NSTEMI differs from UA in that ischemia is severe enough to produce myocardial necrosis. ACS is multifactorial disease and the predominant cause of ACS, in more than 90% of patients is atheromatous plaque rupture, fissuring, or erosion of an unstable atherosclerotic plaque. This exposes thrombogenic matrix proteins and promotes platelet accumulation and local fibrin generation. With repeated cycles of plaque injury and thrombus formation, progressive stenosis of vessel lumen occurs, leading to marked reduction in blood flow and transient tissue ischemia (UA) or total thrombotic occlusion of artery and tissue infarction (AMI) [2]. Vascular homeostasis protects body from these changes and is maintained by inhibiting vascular tone, platelet aggregation and inflammation. These functions are performed by synthesizing a molecule called Nitric oxide ($NO^{\cdot}$). Vasodialatory function of $NO^{\cdot}$ helps in maintaining vascular homeostasis. $NO^{\cdot}$ is synthesized from L- arginine by enzyme nitric oxide synthase (NOS). L-arginine is also a substrate for enzyme arginase. Thus these two enzymes compete with each other for the substrate. Increased arginase decreases NO production and diverts L-arginine to form ornithine. Ornithine forms polyamines and proline which promote aberrant vessel wall remodeling and neointima formation leading to atherosclerosis (Fig. 1). Increased activity of arginase indicates low activity of NOS and thus less production of NO [3].

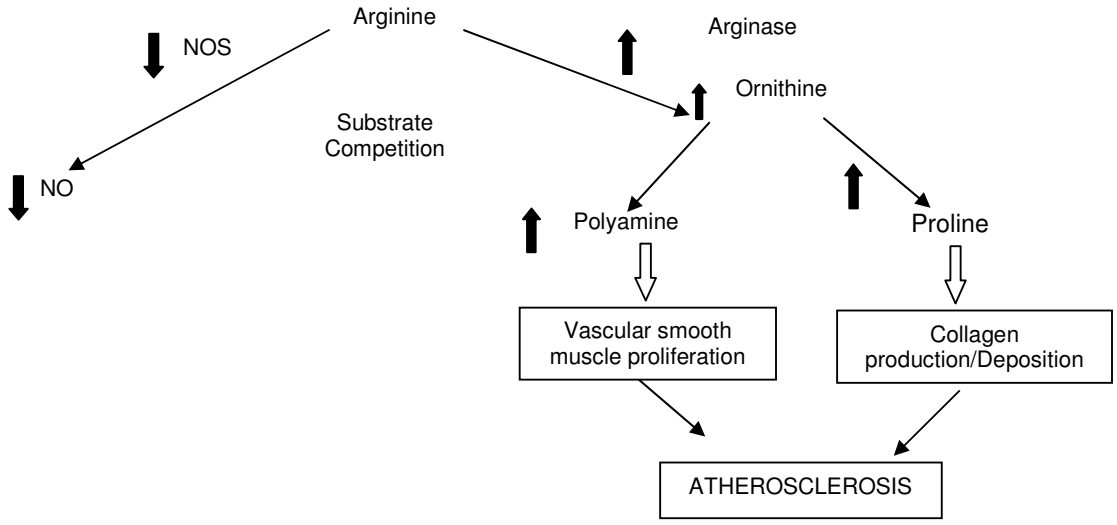

Fig . 1. Role of arginine in atherosclerosis

Thus arginine is related to $NO^{\cdot}$ production and decreased $NO^{\cdot}$ production is directly related to vasodilatation. This relationship led us to think that arginase could be marker of ACS. In addition to this, this can be a additional biomarker available for diagnosing the condition. The diagnosis of ACS is based on clinical symptoms, electrocardiographic (ECG) changes and characteristic pattern of changes in some serum enzymes like aspartate transaminases (AST), creatine kinase (CK-MB), lactate dehydrogenase etc. Keeping these facts in mind it seems that arginase can be a good marker of ACS but there is paucity in the literature regarding relation between increased arginase activity and ACS. So, the present study was undertaken to determine the levels of arginase and its effectiveness over traditional enzymes like AST and CK-MB in ACS so as to use arginase enzyme as a marker.

2. MATERIALS AND METHODS

The study was conducted after Institutional ethical approval and with prior consent of the patients. The study comprised of clinically diagnosed 60 patients of 40 to 70 year age group irrespective of sex: 30 of UA and 30 of MI. 30 age and sex matched healthy individuals without any medication were studied as controls:

2.1 Inclusion Criteria

The study group included patients with chest pain, breathlessness, ECG changes.

2.2 Exclusion Criteria

The patients with diabetes, hypertension, valvular heart disease, pericarditis, chest pain due to respiratory or gastrointestinal cause, alcoholics, smokers, psoriasis, arthritis, sickle cell disease, cancer, on medications and having renal, liver and infectious diseases were excluded from the studies.

The individuals under study were selected after clinical examination by cardiologist on the basis of ECG reports and as per Table 1. They were grouped as follows.

Group I: 30 age and sex matched healthy individuals without any medications were studied as controls.

Group II (UA): Includes patients with ischemic chest pain with recently increased frequency and rest pain. ECG changes show ST depression and T depression.

Group III (MI): Includes patients with ischemic chest pain. ECG changes show STEMI.

Table 1. Shows different parameters used for selecting patients among different groups

Parameters	Group I (n=30)	Group II (n=30)	Group III(n=30)
Age: Mean±SD (years)	48.90±7.05	48.5±15.67	49.26±7.63
Gender	18M/12F	16M/14F	18M/12F
Alcoholic	Zero	Zero	Zero
Smokers	Zero	Zero	Zero
Tobacco	Zero	Zero	Zero
Diabetes	Zero	Zero	Zero
Hypertension	Zero	Zero	Zero
Medication	Zero	Zero	Zero

The blood sample of selected patients was immediately collected after ECG report.

5ml blood sample was collected by venipuncture from individuals under study. The samples were allowed to clot. After 1 hour serum was separated by centrifugation at 2500 rpm for 15 min at room temperature and used to estimate levels of CK-MB [4], AST [5] and arginase [6] within 24 hr of collection. The activity of CK-MB was estimated using kit from Biolab Diagnostics PVT Ltd. The activity of AST was estimated using kit from Transacia Biomedicals PVT Ltd. The activity of arginase was estimated spectrophotometrically and the required chemicals were purchased from Sigma chemicals.

The results were calculated using Microsoft Office Excel 2010 and Graph Pad Prism software version 5.01. The collected data was analyzed by applying unpaired t test and one way ANOVA followed by Bonferronis post test. $P<0.05$ was considered to be statistically significant, $P<0.001$ as highly significant and $P>0.05$ as nonsignificant.

3. RESULTS

Table 2 shows activities of AST, CK-MB and Arginase in groups I,II,III. It was observed that activities of AST, CK-MB and arginase were increased significantly ($P<0.001$) in patients with group III [7]. However this increase was not significant in patients with group II ($P>0.05$).

Table 2. Shows activities of AST, CK-MB and Arginase in groups I,II,III

Group	AST(IU/L)	CK-MB (IU/L)	Arginase(IU/L)
I	19.11±6.18	12.62±5.78	2.20±0.86
II	20.89±5.11	14.35±3.43	2.38±0.35
III	*77.29±14.44	*122.87±21.89	*9.48±1.11

P<0.001- highly significant

4. DISCUSSION

India has the highest burden of ACS in the world. ACS results primarily from diminished myocardial blood flow secondary to an occlusive or partially occlusive coronary artery thrombus. The present study was carried out to find effectiveness of Arginase activity as a marker of ACS. The activity of arginase was compared with that of AST and CK-MB in UA and MI (Table 2).

The results of arginase activities were comparable with that of conventional enzyme markers of ACS i.e. serum AST [8,9] and CK-MB [10-12]. In addition to this, increased activities of arginase reflect the status of $NO^{\cdot}$ as arginase inhibits production of NO [13,14] a vasodilator. This is achieved via several potential mechanisms, including competition with NOS for L-Arginine, uncoupling of NOS resulting in generation of $NO^{\cdot}$ scavengers, superoxide and peroxynitrite, repression of translation and stability of iNOS protein, inhibition of iNOS via generation of urea and by sensitization of NOS to its endogenous inhibitor asymmetric dimethyl L-arginine [15].

In present study, there was no significant increase in activities of CK-MB, AST and arginase in patients of group II whereas a highly significant increase in activities was found in patients with group III ($p<0.001$). There was no correlation observed between CK-MB and AST with arginase in patients of group II whereas a strong positive correlation was found between CK-MB and AST with arginase in group III patients ($P<0.001$).This indicates that like AST and CKMB, arginase activity was also increased. Thus it may be helpful in diagnosis of ACS.However unlike other two,increased activities of arginase reflects $NO^{\cdot}$ status and thus indirectly endothelial functions.

Now a days enzyme arginase is considered as drug targets in MI as arginase inhibitors inhibit arginase and reciprocally elevate levels of $NO^{\cdot}$; a vasodilator. Thus it helps in restoring endothelial functions and reducing vascular plaque burden. Hence arginase could be an important target in atherosclerosis therapy [16-20] and its inhibition provides protection against myocardial injury [21]. This also supports our findings that arginase could be helpful in early diagnosis of ACS and its progression. Additionally it may be used as a prognostic factor for broad population studies.

It was a pilot study and followup study with large sample size will be done. A comparitive study can be carried out to check the usefulness of nonenzymatic cardiac marker like troponin and enzymatic cardiac marker like arginase.

5. CONCLUSION

Arginase activity increases in MI and not in UA. Hence it can be a useful, marker, to differentiate between UA and MI.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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