Original Article

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Biochemistry Section

Ratio Of Serum Asymmetric Dimethyl Arginine (ADMA)/ Nitric Oxide in Coronary Artery Disease patients

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ABSTRACT

Background: Coronary artery disease (CAD) is the leading cause of mortality and morbidity in the world. Current predictions estimate that by the year 2020 cardiovascular diseases, notably atherosclerosis will become the leading global cause of the total disease burden. Atherosclerosis of the Coronary artery causes myocardial infarction and angina pectoris. Endothelial Nitric oxide (NO), released by the intact and healthy endothelium plays a very important role in the maintenance of vascular tone and structure. Decreased NO level leads to endothelial dysfunction is an initial event in the atherosclerosis. Endogenous Asymmetric dimethylarginine (ADMA) is a structural analog of L-arginine, competitively inhibits the enzyme NO synthase and thus decreases the NO level.

Aim: To study the ratio of serum ADMA / NO as a marker of severity of CAD.

Materials and Methods: The study comprises of 60 patients of CAD diagnosed by coronary angiography. We divided them into two Groups according to percentage of atherosclerotic block, Group A (71% and more block, n=30) and Group B (40% - 70% block, n=30). We measured serum ADMA, serum NO and calculated ADMA/ NO ratio. Results were compared with 30 healthy age and sex matched controls.

Serum ADMA was determined by reverse phase high performance liquid chromatography. Serum NO was measured by cadmium reduction method. Statistical analysis of data analysis was done using the SPSS (Statistical Package for the Social Science) Version 11 for window.

Results: Serum ADMA was correlated positively with the presence and severity of CAD and inversely related with the serum NO levels. Serum ADMA / NO ratio was statistically significant in CAD patients with atherosclerotic block 71% and above (Group A) but ratio was not significant in Group B (block 40% - 70%).

Conclusion: Serum ADMA/ NO ratio can be the better predictive marker for the severity of the CAD where patient is at the risk of angina pectoris or myocardial infarction due to the extent of coronary atherosclerotic block than individual serum ADMA levels .

Keywords: Asymmetric dimethyl arginine(ADMA), Coronary artery disease(CAD), Nitric oxide (NO)

INTRODUCTION

Coronary artery disease (CAD), which can cause occlusion of one or more coronary arteries, is the leading cause of morbidity and mortality in developing countries [1]. Atherosclerosis is the main cause of CAD. It is an inflammatory disease which involves interaction of immune system with metabolic risk factors to initiate, propagate and activate lesion in the coronary arterial tree [2]. Atherosclerosis of the Coronary artery causes angina pectoris & the myocardial infarction (MI). [3]. Atherosclerotic plaque in CAD causes narrowing of coronary vessel called stenosis leading to subsequent decrease in blood flow through the affected vessel, reducing the coronary blood flow dramatically which causes myocardial ischemia leading to angina pectoris or MI [4].

Intact healthy endothelium plays a major role in vascular homeostasis which secretes number of regulatory substances to maintain the vascular tone. Damaged endothelium cannot perform this crucial task and it becomes dysfunctional which is the initial event on the long path towards atherosclerosis [5-6]. Among these regulatory substances Nitric Oxide (NO) is most important vasodilator substance released by the endothelium [3-7] which also inhibits the adhesion and aggregation of platelets, adhesion of monocytes and leukocytes to the endothelium, vascular smooth muscle cell proliferation and low density lipoprotein (LDL) oxidation [8-10]. Abridged NO bioavailability compromises all these functions which lead to initiation and progression of atherosclerosis [10]. Nitric oxide, an 'endogenous anti-atherogenic molecule',is produced from L-arginine by endothelial Nitric Oxide synthase (eNOS) which requires molecular oxygen and various cofactors. [9]. Any condition which reduces endothelial NO production possibly will consequently promote atherosclerosis [11].

Asymmetric dimethylarginine (ADMA), being structural analogue of L-arginine acts as an endogenous competitive inhibitor of eNOS and it contributes to endothelial dysfunction [12-13]. Various studies have revealed that high level of baseline ADMA is independently prognostic of adverse outcomes in a variety of populations including stable angina , unstable angina and established coronary artery disease [14-16]. However, little is known about the relationship of serum ADMA and percentage of the atherosclerotic block in CAD patients. Taking into account the promising role of ADMA & NO in cardiovascular diseases we studied serum ADMA/NO ratio in CAD patients to assess the severity (percentage) of atherosclerotic block.

MATERIALS AND METHODS

The present study is a cross-sectional study. It has been carried out in B.J.Goverment Medical College and Sassoon General Hospital, Pune, India. The study comprised of 60 CAD patients diagnosed by coronary angiography on admission to the hospital with the complaints suggestive of angina or MI. The selected CAD patients (n=60) were subdivided into two Groups as Group A (n=30) included patients with 71% atherosclerotic block or more than that. Group B (n=30) included patients with block 40%-70%. (For our convenience

Coronary segment with the highest block was taken into the consideration for division irrespective of the site of the blockage and number of segments involved.) Group C (n=30) included healthy age and sex matched individuals as controls. Blood samples were collected on admission and allowed to clot at room temperature and then centrifuged at 2500rpm for 15 minutes. Serum was stored in two separate aliquots at -80°C until analysis.

Inclusion criteria: Patients with clinically relevant CAD which was defined as the occurrence of ≥1 stenosis of ≥40% in ≥1 of 15 coronary segments.

Exclusion criteria- We excluded individuals with stenosis <40%, subjects suffering from Diabetes Mellitus (DM), Chronic Kidney Disease and those who were on Antihypertensive drugs or Statin therapy from the study.

The study protocol was approved by the Ethical Committee of the Institute. Informed written consent was obtained from all the study subjects enrolled in the study.

Determination of Serum ADMA by Reverse Phase High performance liquid chromatography (HPLC): Chemicals were purchased from Merckand Sigma.

Sample clean up was performed by solid phase extraction using vacuum manifold with a capacity of 12 columns & N-Monomethyl L-arginine (L-NMA) was used as internal standard. Cation exchange columns used for solid phase extraction of ADMA from serum samples were supplied by GRACE Ltd. India. The recovery of ADMA was found to be >80%. After derivatization with o-phthaldialdehyde reagent containing mercaptoethanol, ADMA was separated by gradient reverse phase HPLC with fluorescent detection set at excitations and emission wavelengths of 340 and 455 nm, respectively. The stable derivatives were separated with near baseline resolution. All separations were performed at 30°C at a flow rate of 1.1 ml/min. Low limits of detection for ADMA were 50ng/ml [17].

Serum NO by cadmium reduction method

Nitrate in serum was assayed by the cadmium-reduction method; after samples were deproteinized with Somogyi reagent, the nitrate was reduced by Cu-coated Cd in glycine buffer at pH 9.7 (2.5 to 3 g of Cd granules for a 4-mL reaction mixture) which took 90 minutes for the reduction. The nitrite produced was determined by diazotization of sulfanilamide and coupling to naphthylethylene diamine. Detection limits in urine or serum were 10 to 25umol/L [18].

STATISTICAL ANALYSIS

Data analysis was done using the SPSS (Statistical Package for the Social Science) Version 11 for window. The ANOVA test was used to significant difference between Groups, Tukey's test was used for inter Group comparison and correlation between variables in each Group. A probability value of 0.05 was accepted as the level of statistical significance.

Parameters	Group A	Group B	Group C	f-Value	p-value
	Mean ± SD (n=30)	Mean ± SD (n=30)	Mean ± SD (n=30)		
ADMA (umol/L)	0.96 ± 0.08	0.64 ± 0.08	0.41 ± 0.09	300.84	<0.0001
NO (umol/L)	22 ± 8.99	64.67 ± 10.18	98.5 ± 12.59	385.25	<0.0001

Group A Vs Group B: p<0.0001 Group. A Vs Group C: p<0.0001
Group B Vs Group C: p<0.0001
There is highly significant rise in serum ADMA level and a significant decrease in serum NO in Group A as well as Group B as compared to Group C (p-value <0.0001). The rise in serum ADMA along with fall in serum NO is also highly significant in Group A as compared to Group B (p-value <0.0001).

[Table/Fig-1]: Comparison of serum ADMA and serum NO in study Groups

Parameters	Group A	Group B	Group C	F Value	P Value
	Mean ± SD (n=30)	Mean ± SD (n=30)	Mean ± SD (n=30)		
ADMA/NO	0.05 ± 0.02	0.01 ± 0.003	0.004 ± 0.0008	100.98	<0.0001

Group A Vs Group B: p<0.0001 Group A Vs Group C: p<0.0001
Group B Vs Group C : Not Significant
Highly significant rise in serum ADMA /NO ratio is seen in Group A as compared to Group B & C .(P value <0.0001). ADMA / NO ratio is raised in Group B as compared to Group C but rise is not statistically significant.

[Table/Fig-2]: Comparison of serum ADMA/ NO ratio in study Groups

RESULTS

[Table/Fig-1] depicts mean ± SD serum ADMA and serum NO levels in Group A,B and C.

In this study, we found serum ADMA levels were raised significantly in study Groups at the same time significant fall in serum NO levels were seen in the study Groups as compared to controls(P <0.0001). In order to study how increased serum ADMA affects synthesis of NO we calculated ADMA/NO ratio in [Table/Fig-2]. Highly significant rise in serum ADMA /NO ratio is seen in Group A as compared to Group B & C.(p-value <0.0001). ADMA / NO ratio are raised in Group B as compared to Group C but rise is not statistically significant.

DISCUSSION

Role of NO in vascular homeostasis is known to all of us. ADMA is structural analogue of l-arginine, a substrate for endothelial nitric oxide synthase enzyme. ADMA competitively inhibits the synthesis of Nitric Oxide & play crucial role in the initiation of endothelial dysfunction [12-13]. In the present study we found statistically significant decrease in serum NO level in Group A and Group B as compared to Group C (p-value <0.0001). A significant decrease in serum Nitric Oxide was also observed in Group A as compared to Group B (p-value <0.0001) [Table/Fig-1].In addition to NO we estimated serum ADMA levels. We observed a significant increase in serum ADMA in Group A and Group B as compared to Group C as well as a significant increase in Group A as compared to Group C (P<0.0001) [Table/Fig-1]. This suggests that raised ADMA might be competitively inhibiting NO synthesis being structural analogue of L-Arginine.

These findings were supported by the study of Boger RH et al., who reported that elevated ADMA levels were associated with reduced NO production in hypercholesterolemic subjects and in atherosclerotic patients [19].

In vitro experiments with purified NO synthase isoforms, Vallance et al., reported that inhibition of macrophage NO synthase is dependent on ADMA concentration [12].

Our results are also consistent with Andreas Meinitzer et al., they reported in their study that ADMA concentration predicts all-cause and cardiovascular mortality in individuals with CAD independently of established and emerging cardiovascular risk factors. In the same study they found that unadjusted ADMA was higher in the 2nd, 3rd, and 4th strata compared with the lowest stratum, which were stratified according to the severity of the CAD [13]. So far few prospective studies have highlighted the predictive value of ADMA for total mortality and cardiovascular mortality [19-22].

But there is paucity of data about the association of ADMA and NO with respect to severity of atherosclerotic block in CAD. We observed that ADMA levels were increased with increasing severity of the CAD with concomitant fall in NO [Table/Fig-1]. ADMA

competitively inhibits NO synthesis in a concentration dependent manner. Another fact that impairs NO function is uncoupling of endothelial Nitric Oxide synthase catalytic activity which is observed under the condition of L-arginine deficiency by structural analog ADMA [9]. Various studies supports our finding that ADMA inhibits NO production in concentration dependent manner [19,23].

The persons with atherosclerotic block > 40% but less than 80% are at the risk of stable angina while those with stenosis more than 80% have compromised coronary blood flow at rest resulting into unstable angina or total occlusion of coronary artery leading to the MI [4-6] Due to the inverse relation of ADMA & NO we calculated ADMA /NO ratio as a predictive marker of severity of CAD. We found ADMA/NO ratio is raised significantly in Group A as compared to Group B and C (p-value<0.0001). But ratio is not significant in Group B as compared to Group C [Table/Fig-2]. This shows that ADMA/NO ratio has positive relationship with severity of atherosclerotic block in CAD patients. The significance of the ratio increases as atherosclerotic block exceeds 71% and above (Group A) where ischemia can occur at rest as compared to those who are having stenosis >40% but less than 70% (Group B) as well as normal persons (Group C).

LIMITATIONS

These findings need to be confirmed in larger study Groups. We did not differentiated CAD patients into stable Angina and Acute Coronary Syndrome Groups which might show some differences in the Serum ADMA levels. Also controls were selected without angiography this limits the efficacy of the results.

CONCLUSION

In the present study we found highly significant rise in the serum ADMA and concomitant decrease in NO levels in CAD patients (Group A & B) as compared to controls. Highly significant rise was also seen in the serum ADMA/NO ratio in Group of patients with atherosclerotic block 71% and more (Group A) as compared to Group B and Group C. While ratio was not significant in Group B as compared to Group C. Our result concludes that serum ADMA being structural analogue of L-Arginine, inhibits serum nitric oxide synthesis in concentration dependent manner. This aggravates the atherosclerotic progression increasing the severity of atherosclerotic block in CAD. Also, ADMA / NO ratio indicates that it can be a predictive marker of severity of CAD which increased significantly in Group A where the patient is at the risk of MI or unstable angina as compared to Group B & Group C. It is a better marker of severity of CAD than individual serum ADMA or serum NO values which are raised significantly in both Group A & B as compared to Group C. These findings allow the stratification of patients with respect to the risk for subsequent future cardiovascular events.

REFERENCES

[1] Sheridan PJ, Crossman DC. Critical review of unstable angina and non-STelevation myocardial infarction. *Postgraduate Med J*. 2002; 78: 717–26.

[2] Göran K Hansson M.D, Ph.D. Review Article Inflammation, Atherosclerosis, and Coronary Artery Disease. *N Engl J Med*. 2005; 352:1685-95.

[3] Peter Libby .The Pathogenesis , Prevention and Treatment of Atherosclerosis 17th edition Harrison's Principles Of Internal Medicine. 1501-09.

[4] Elliot M. Antman, Andrew P. Selwyn, Eugene Braunwald, Joseph Loscalzo. *Ischemic Heart Disease Harrison's Principles Of Internal Medicine.* 17th Edition 1514-27.

[5] Christophe depre/ Stephen F. Vatner/ Garrett J. Gross Coronary Blood Flow and Myocardial Ischaemai .*Hurst's The Heart. 12th Edition* . 1259-76.

[6] Juan Jose Badimon. Coronary Thrombosis:Local & systemic Factors. *Hurst's The Heart 12th Edition* 1245-58.

[7] V. Schächinger, A.M. Zeiher. *Atherosclerosis-associated endothelial dysfunction. Nov2000;89(9):* pp IX70-IX74.

[8] Mohsen Kerkeni, Faouzi Addad, et al. Hyperhomocysteinemia, Endothelial Nitric Oxide Synthase Polymorphism, and Risk of Coronary Artery) Disease; *Clinical Chemistry.* 2006; 52: 153-58.

[9] The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor. *Oxford Journals, Medicine.Cardiovasc Res.* 2003; 59(4): 824-33.

[10] Peter J Kuhlencordt MD, Robert Gyurko PhD, Fred Han BS, Marielle Scherrer-Crosbie MD, et al. *Accelerated Atherosclerosis, Aortic Aneurysm Formation, and Ischemic Heart Disease in Apolipoprotein E/Endothelial Nitric Oxide Synthase Double-Knockout Circulation.* 2001; 104: 448-54.

[11] Maurício Batista Paes Landim; Antônio Casella Filho; Antônio Carlos Palandri Chagas et al. Asymmetric dimethylarginine (ADMA) and endothelial dysfunction: implications for atherogenesis. *Clinics São Paulo.* 2009;64(5):471–78 .

[12] Vallance P, Leone A, Calver A, Collier J, Moncada S,et al Accumulation of an endogenous inhibitor of NO synthesis in chronic renal failure. *Lancet.* 1992;339:572-75.

[13] Andreas Meinitzer ,Ursula Seelhorst, et al. Asymmetrical Dimethylarginine Independently Predicts Total and Cardiovascular Mortality in Individuals with Angiographic Coronary Artery Disease (The Ludwigshafen Risk and Cardiovascular Health Study) . *Clinical Chemistry.* 2007; 53 (2): 273-83.

[14] Tse-Min Lu Yu-An Ding ,Shing-Jong Lin, et al. Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention. *Eur Heart J.* 2003;24(21): 1912-19.

[15] Renate Schnabel, Stefan Blankenberg, et al. Asymmetric Dimethylarginine and the Risk of Cardiovascular Events and Death in Patients With Coronary Artery Disease Results from the AtheroGene Study. *Circulation Research.* 2005; 97: e53-e59.

[16] Tanja K Krempl, Renke Maas, Karsten Sydow, Thomas Meinertz, et al. Elevation of asymmetric dimethylarginine in patients with unstable angina and recurrent cardiovascular events. *Eur Heart J*. 2005; 26(18):1846-51.

[17] Kai-Min Chu, Pei-Wei Huang, and Li-Heng Pao, et al.Determination of Arginine, Asymmetrical Dimethylarginine, and Symmetrical Dimethylarginine in Human Plasma by High Performance Liquid Chromatography. *J Med Sci.* 2003;23(4):201-06.

[18] Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. *Clin Chem*. 1990 Aug;36(8 Pt 1):1440-43.

[19] Rainer H Böger MD, Stefanie M Bode-Böger MD, et al .Asymmetric Dimethylarginine (ADMA): A Novel Risk Factor for Endothelial Dysfunction Its Role in Hypercholesterolemia. *Circulation.* 1998; 98: 1842-47.

[20] Marianne Zeller,Claudia Korandji, et al. Impact of Asymmetric Dimethylarginine on Mortality After Acute Myocardial Infarction. *Arteriosclerosis, Thrombosis, and Vascular Biology.* 2008; 28: 954-60 .

[21] Valkonen VP, Paiva H, Salonen JT, Lakka TA, Lehtimaki T, Laakso J, et al. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. *Lancet*. 2001;358:2127-28.

[22] Cavusoglu E, Ruwende C, Chopra V, Yanamadala S, Eng C, Pinsky DJ,et al. Relationship of baseline plasma ADMA levels to cardiovascular outcomes at 2 years in men with acute coronary syndrome referred for coronary angiography. *Coron Artery Dis*. 2009;20(2):112-17.

[23] Rainer H Böger MD, et al Biochemical Evidence for Impaired Nitric Oxide Synthesis in Patients With Peripheral Arterial Occlusive Disease. *Circulation.* 1997; 95: 2068-74.

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