Original Research Article

Serum asymmetric dimethylarginine, a novel risk marker of coronary artery disease

G Lakshmi[1], Jothi Malar R[2],*

[1]Dept. of Biochemistry, Panimalar Medical College Hospital and Research Institute, Chennai, Tamil Nadu, India
[2]Dept. of Biochemistry, Sri Ramachandra Medical College and Research Institute, Chennai, Tamil Nadu, India

ABSTRACT

Background: Coronary artery disease (CAD) is a chronic disease and a global health problem, affecting one-third of the world's population. Recently it has been found that the traditional risk factors of CAD do not account for the total risk. One of the novel risk markers of CAD is Asymmetric dimethylarginine (ADMA), which inhibits the enzyme nitric oxide synthase (NOS), which is required for the synthesis of nitric oxide (NO), a potent vasodilator. Increased ADMA level causes decrease in NO level which will lead to endothelial dysfunction, an underlying causative mechanism for CAD development. ADMA is found to be an independent cardiovascular risk factor. Therefore ADMA may become an additional biochemical parameter to identify those patients who are at higher risk for developing CAD, besides the traditional parameters used.

Aim: To study the association between serum ADMA level with coronary artery disease.

Materials and Methods: The study design is a case-control study. 80 subjects from the south Indian population between 30 to 60 years of age, of both the sexes were included in the study. Out of them, 40 individuals were angiographically proven cases of CAD from the department of cardiology and 40 healthy individuals from the master health check up department. Various parameters like lipid profile, serum ADMA, blood pressure and BMI were measured in both the groups. The data were collected and analyzed.

Result: The ADMA level was found to be significantly elevated in the case group compared with the age matched healthy controls (p value < 0.05).

Conclusion: There is a positive correlation between serum ADMA level and coronary artery disease.

1. Introduction

Coronary artery disease (CAD) is a most common cardiovascular disease with increased global burden. It is a chronic disease due to atherosclerosis of coronary arteries.[1] Either one or more than one coronary arteries may be involved. With time this may lead tomyocardial infarction(MI), a complication of CAD which occurs due to the rupture of plaques in the coronary arteries and formation of tiny clots which will obstruct the blood flow to the heart, leading to sudden death or severe hemodynamic detoriation.[2]

According to recent surveys, approximately one-third of world's population die due to CAD and 80% of these deaths occur in the developing countries.[2] CAD has become a global health problem. In 2013, 17.3 million deaths worldwide were related to CAD, as estimated by "The global burden of disease study".[3,4] Recent studies predicts that globally the leading cause of total disease burden will be due to atherosclerosis by the year 2020.[5]

In India there is rising burden of CAD and studies conducted have reported rising trends in the levels of risk factors contributing to atherosclerosis such as diabetes, hypertension and metabolic syndrome.[6] All these further causes burden to the Indian economy. Apart from the higher mortality rate, CAD manifests a decade earlier in

* Corresponding author.
E-mail address: drjothimalar@gmail.com (J. Malar R).

Indian population compared to the rest of the world.[7] This results in tremendous loss of productive working years of life.[8] These points further highlights the need for aggressive strategies for the control and prevention of CAD in India.[6,7]

Globally, many epidemiological studies have been conducted to establish the various risk factors for CAD development.[2] According to these studies, the traditional risk factors such as hypertension, diabetes, obesity, dyslipidemia and cigarette smoking were the top five causes of death globally but they do not contribute to the total risk. Therefore additional risk markers which predicts the risk of cardiovascular events is the need of the hour.[9]

Asymmetric dimethyl arginine (ADMA) is one such biomarker which has emerged as a novel cardiovascular risk marker. It is a post- translationally modified form of amino acid L-arginine. ADMA works by decreasing nitric oxide (NO) level, which is a potent vasodilator, by inhibiting nitric oxide synthase (NOS), an enzyme required for NO synthesis.[10] Decreased NO level causes endothelial dysfunction, which is a underlying causative mechanism for CAD development.[11] Therefore it was shown that increased level of ADMA will cause endothelial dysfunction, which paves the way for the development of CAD.[10] ADMA is also an independent cardiovascular risk factor and it is also useful in patients who are already diagnosed with CAD.[9] CAD patients with higher ADMA level are at increased risk to experience future major cardiovascular events.

Therefore ADMA may be used as an additional biochemical parameter to identify those patients who are at higher risk of developing cardiovascular events, besides the traditional parameters used nowadays.

2. Materials and Methods

2.1. Patient's selection and study design

The study was conducted at Sri Ramachandra Medical college and Research Institute, Chennai. In this case-control study, 80 subjects from the south Indian population between the age group of 30 to 60 years, of both the sexes were included.

Out of the 80 subjects, 40 individuals were angiographically proven cases of CAD, from the Cardiology department. Individuals with preexisting renal or hepatic insufficiency or with any other cardiac, acute or chronic illness were excluded from the case group.

The control group comprised of 40 healthy individuals who underwent master health checkup with no history of diabetes, hypertension, dyslipidemia, thyroid disorders, CAD/ any other cardiac illness, renal disease, hepatic insufficiency or any other acute or chronic illness.

2.2. Ethical clearance

Ethical clearance for this study was obtained from Institutional Ethics Committee (IEC) of Sri Ramachandra

Medical College & Research Institute and informed consent was obtained from all the participants of the study.

2.3. Sample collection

Blood samples were collected in a gel tube from the study participants. After 30 minutes the samples were centrifuged for 10 minutes at 3500 rpm. The serum samples were aliquoted in 2 ml polypropylene tubes and were labeled with clear sample ID's. Estimation for lipid profile was done on the same day. Samples for ADMA estimation were stored at -20°C.

Body mass index (BMI) of the study participants was calculated as weight/height^2(Kg/m^2). Systolic and diastolic blood pressure was measured.

2.4. Laboratory measurements

Serum ADMA was measured using Human ADMA ELISA kit which was procured from the Sincere Biotech Co., Ltd. The calibrators were used to construct a calibration curve from which the concentration of ADMA in the test specimens was obtained using ELISA reader software program.

The lipid profile was analyzed using automated analyzer (Siemens Advia 1800).

2.5. Statistical analysis

Statistical analysis was performed using SPSS software version 17.0. Data was expressed as mean $\pm$ SD. Independent sample t-test and ANOVA were performed to check for the statistical significance for differences in mean between the groups.

3. Results

The baseline characteristics of the groups are presented in Table 1.

Table 1: Baseline characteristics of the study population

Variables	Control Group (N= 40)	Case Group (N=40)	p-value
Age (years)	45 $\pm$ 7	46 $\pm$ 8	0.635
BMI(Kg/m2)	24 $\pm$ 3	27 $\pm$ 3	0.008*
SBP(mmHg)	113 $\pm$ 8	133 $\pm$ 14	0.000*
DBP(mmHg)	73 $\pm$ 6	87 $\pm$ 7	0.000*
Total cholesterol(mg/dL)	144 $\pm$ 20	184 $\pm$ 36	0.0001*
TGL(mg/dL)	93 $\pm$ 29	172 $\pm$ 80	0.0001*
HDL(mg/dL)	43 $\pm$ 11	36 $\pm$ 6	0.002*
LDL(mg/dL)	87 $\pm$ 12	129 $\pm$ 34	0.0001*

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TGL: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein p-value<0.05: Statistical significance*

Table 2: Comparison of ADMA level between control & case group

Paramater	Control Group N = 40	Case Group N = 40	p-value	Statistical Significance
ADMA(ng/mL)	91.26±13.91	154.14±17.81	0.0001	HS

HS- Highly significant

3.1. Comparison of serum ADMA level between control & case group

The mean value of serum ADMA in the control group was 91.26 ± 13.91 ng/mL and in the case group 154.14 ± 17.81 ng/mL. The difference in the values was found to be statistically significant with the p-value of 0.0001.
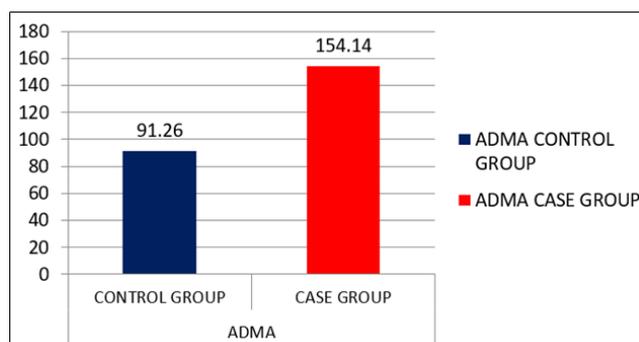
Fig. 1: Bar diagram showing the mean ADMA level between control group & case group

Table 3: Pearson correlation of ADMA with lipid profile, blood pressure and BMI in case group

Parameter	r-value	p value
Total cholesterol	0.26	0.10
TGL	0.19	0.24
LDL	0.26	0.10
HDL	-0.51	0.75
SBP	0.18	0.22
DBP	0.04	0.18
BMI	0.15	0.20

Table 3 shows correlation of ADMA with lipid profile parameters (Total cholesterol, TGL, LDL, HDL), blood pressure and BMI in the case group. ADMA shows weak positive correlation with Total cholesterol, TGL, LDL, SBP, DBP & BMI but there is no statistical significance. With HDL, ADMA shows moderate negative correlation which is not statistically significant.

4. Discussion

CAD prevalence has increased dramatically worldwide and in the recent years there is increased need for identifying risk factors for early detection of atherosclerosis and CAD.[12] Even though the known atherogenic risk factors such as obesity, Type 2 diabetes mellitus, hypertension, dyslipidemia and smoking are preventable, the global burden of CAD still remains high.[13]

Therefore many novel biochemical risk markers such as highly sensitive C-reactive protein (hs-CRP), homocystein, fibrinogen, soluble intercellular adhesion molecule 1, small dense LDL, P-selectin, ADMA, etc., have been identified as markers of early detection of CAD and some of these markers are also helpful as prognostic indicators of CAD.[12,14]

Among these markers ADMA was shown to have causative role in endothelial dysfunction by many studies.[15] Many studies have shown that all the major risk factors of atherosclerosis disturb the normal function of the vascular endothelium.[11,16] The vascular endothelium plays many important functions including vascular tone maintenance, control of cellular adhesion, regulation of thrombo resistance, vessel wall inflammation etc., Therefore disturbance in the vascular endothelial function leads to atherosclerotic plaque formation.[17]

Nitric oxide (NO) is required for the normal functioning of vascular endothelium. NO is produced from the amino acid L-arginine catalyzed by the enzyme Nitric oxide synthase (NOS).[9] ADMA is derived from the degradation of methylated proteins in the course of protein turnover. ADMA inhibits the enzyme NOS, leading to decreased synthesis of NO. This in turn leads to vascular dysfunction which is an important early step in CAD development.

Many studies have shown the role of elevated level of ADMA in disorders of endothelial dysfunction such as hypertension, diabetes mellitus, dyslipidemia and atherosclerosis.[15] Globally studies which have been conducted in different groups have shown that there is increased ADMA level observed in CAD patients than healthy individuals. These studies have also found that ADMA is a independent risk marker of atherosclerosis.[12] It was also shown to predict the future MI attacks in already diagnosed CAD patients.[18] Therefore measurement of ADMA in biological fluids may be a promising tool in early detection of CAD and may also to predict the adverse cardiovascular events in patients with CAD.

Schulze F and Maas R et al. conducted a study in order to determine the reference range of ADMA using ELISA, and they established the reference range for ADMA in plasma as 0·36–1·17 μmol/L (which is equivalent to 74.44- 235.42 ng/ml).[19]

In this study the serum ADMA was measured among the case and the control subjects to find out the association between ADMA and CAD. The correlation of ADMA with

lipid profile parameters, blood pressure and BMI was also studied. The mean and standard deviation was measured for all the parameters to find out the statistical significance.

In this study the case group had a statistically significant higher ADMA level when compared to the control group (p-value of 0.0001). In the study conducted by Friedrich Schulze et al. differences in ADMA plasma levels between patients with CAD and healthy individuals were analyzed and they found that cases had higher ADMA plasma levels than controls thus supporting this study finding.[9] The result of this study corroborated well with the other studies done by, Kielstein JT et al.[20] and Achan V et al.[16]

In this study the parameters of lipid profile such as total cholesterol, TGL, HDL and LDL were compared among the control and the case group. The mean values of lipid profile parameters were higher among the case group compared to the controls. The values were found to be statistically significant with the p-value$\leq$0.01 (Table 1). The correlation of ADMA with lipid profile was also done. ADMA shows very weak positive correlation with total cholesterol, TGL and LDL(r-value of 0.26, 0.19, 0.26) but there is no statistical significance. With HDL, ADMA shows negative correlation(r-value:-0.51) but there is no statistical significance seen. A study conducted by Rainer H Boger et al.[11] also showed positive correlation and significant association of ADMA level with dyslipidemia. In this study there was only very weak correlation and no statistical significance seen. A study with larger sample size is required to establish the findings.

In this study the difference in the mean systolic and mean diastolic blood pressure were measured among the case and the control group was found to be statistically significant with the p-value of 0.0001. The ADMA level and the systolic and diastolic blood pressure was found to be weakly positively correlated(r-value: 0.18, 0.04) and there was no statistical significance. The study conducted by Achan V et al. ADMA was shown to cause hypertension and cardiac dysfunction in humans. Similarly in a study conducted by Miyazaki et al. there is a statistically significant association between ADMA level and systolic and diastolic blood pressure were observed with a p-value of <0.0001.[21] In this study there was no statistical significance seen. This may be due to the limited sample size compared to these studies.

Since obesity is a risk factor for CAD and association of increased ADMA in obesity was found out by various studies, BMI was calculated in all the subjects in our study. BMI of the case group is higher compared with the control group and was found to be statistically significant with the p-value of 0.008. ADMA correlated positively(r-value: 0.15) with BMI and there was no statistical significance seen. This may be due to the limited sample size. In a study conducted by Friedrich Schulze et al. a significant association between ADMA level and BMI was seen.[8]

This study has shown that coronary artery disease(CAD) patients had increased ADMA level.

5. Conclusion

ADMA level was higher in angiographically proven CAD patients compared to healthy individuals. Therefore our study suggests that measurement of ADMA may become an additional biochemical parameter to identify the at risk individuals and help in early detection and prevention of future cardiovascular events in these individuals besides the traditional parameters used.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Author biography

G Lakshmi Assistant Professor

Jothi Malar R Professor