

## Asymmetrical dimethyl arginine in type 2 diabetic patients with coronary artery disease

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### Abstract

**Objective:** To compare clinical and biochemical parameters for type 2 diabetic patients having coronary artery disease with controls.

**Methods:** The analytical, cross-sectional study was conducted at Khyber Medical College, Peshawar, Pakistan, for a period of one year between 2010 and 2011, and comprised two groups; Group A had normal controls, while Group B had type 2 diabetic patients with coronary artery disease. Clinical parameters were blood pressure and body mass index. Blood was centrifuged for blood sugar, glycosylated haemoglobin, total cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride and asymmetrical dimethylarginine. SPSS 15 was used to analyse data.

**Results:** Of the 150 subjects, there were 75(50%) each in Group A and Group B, which had the mean age of 50.8±80 years and 53.4±52 years, respectively. Both systolic and diastolic blood pressure and body mass index were raised and an elevated level of serum asymmetric dimethylarginine, fasting blood sugar, total cholesterol, triglycerides, glycosylated haemoglobin was noted among Group B patients ( $p < 0.05$ ).

**Conclusion:** All clinical and biochemical parameters were found raised among diabetic patients with coronary artery disease.

**Keywords:** Type 2 diabetes mellitus, Coronary artery disease, Asymmetrical dimethylarginine, Glycosylated haemoglobin. (JPMA 66: 957; 2016)

### Introduction

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide (NO) synthase, produced by methylation of specific arginine residues of certain cellular proteins and released when hydrolysis of these proteins occurs.<sup>1,2</sup> It is eliminated from the body by renal excretion (20%) and also metabolised (80%) by hydrolytic degradation to citrulline and dimethylamine (DMA) by the enzymatic action of dimethylarginine dimethylaminohydrolase (DDAH).<sup>3,4</sup> Increased concentration of ADMA will compete with L-arginine to be transported into the endothelial cells leading to decreased synthesis of NO and endothelial dysfunction.<sup>5-7</sup>

The prevalence of type 2 diabetes mellitus (T2DM) is increasing, and according to the World Health Organisation (WHO), it will increase up to 300 million by 2025.<sup>4,8</sup> It is silently emerging as an epidemic imposing burden on the healthcare services all around the world because of its microvascular complications (retinopathy, neuropathy and nephropathy) and macrovascular complications (hypertension [HT], deranged lipid metabolism and arteriosclerosis) leading to coronary

artery disease (CAD) and cerebrovascular stroke (CVS).<sup>5,8,9</sup>

CAD accounts for large fraction of morbidity and mortality in T2DM patients.<sup>5</sup> Sixty-five per cent of T2DM patients die of CAD with the affected adults having 2-4 times more risk of developing CAD than non-diabetic patients.<sup>2-4</sup>

The conditions which increase the chances of developing CAD among T2DM patients are called risk factors, according to the American Diabetic Association.<sup>10,11</sup> They include positive family history for CAD, age (>45 years for men, >55 years for post-menopausal women), gender (male>female), BP >140 systolic >90 diastolic, body mass index (BMI) >25Kg/m<sup>2</sup>, hypercholesterolaemia (Low High-density lipoprotein cholesterol [HDL-C] > low-density lipoprotein cholesterol [LDL-C]), triglycerides (TGs) >150mg/dl, fasting blood glucose (FBG) >125mg/dl and raised ADMA levels (>0.6umol/L).<sup>11</sup>

ADMA is considered indicator of endothelial dysfunction and CAD because it is associated with HT, insulin resistance (IR), hyperglycaemia atherosclerosis with raised LDL-C for past two decades. There is a strong relationship between serum ADMA and macrovascular disease in T2DM. It is a potential cause of CAD in diabetics and causes 75% DM-related deaths.<sup>12</sup>

The current study was planned to compare clinical and

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biochemical parameters for T2DM patients with CAD to normal group, and to define the role of ADMA in development of CAD in T2DM patients.

### Patients and Methods

The analytical, cross-sectional study was conducted at Khyber Medical College (KMC), Peshawar, Pakistan, for a period of one year between 2010 and 2011, and comprised T2DM patients divided into two groups: Group A had healthy controls, and Group B had diabetics with positive history of CAD. Group B patients had already been diagnosed with T2DM for at least 3 years and had myocardial infarction (MI) in the last seven days. All the participants were aged between 35 and 65 years. Patients using lipid-lowering therapy or rennin angiotensin system inhibitors (RAS) were excluded. An informed consent was signed by all the participants, and demographic details, complete clinical history, relevant physical examinations and other information was recorded in a pre-prepared structured proforma. Approval of the study was obtained by the institutional ethical review committee. According to WHO guidelines,<sup>13</sup> BMI was taken as 25-29kg/m<sup>2</sup> for both genders. Systolic (SBP) and diastolic blood pressure (DBP) was recorded for all the patients and was marked as hypertensive if above 120/80.

Blood samples were collected by aseptic technique after 12-hour overnight fasting. The blood was centrifuged at 3,000 rpm for five minutes and serum was separated. The serum was collected in Eppendorf tubes which were appropriately labelled and stored at -20°C for further analysis. The samples were analysed for FBG, TG, total cholesterol (TC), HDL-C and ADMA levels. A portion of blood sample was put in ethylenediaminetetraacetic acid (EDTA) bottle for estimation of glycosylated haemoglobin (HbA1c) level.

FBG, TC level, total TG and HDL-C were analysed by the enzymatic colorimetric method with relevant kits (Elitech-Sees, France). HDL-C was determined colorimetrically with another kit (Diasys Holzheim, Germany). According to the principle of colorimetry, a specific colour was obtained for each analyte after treatment with particular reagents. The absorbance of the colour obtained was measured using spectronic-20 with a specific wavelength mentioned in the kits. LDL-C was calculated using Friedwald formula,<sup>14</sup> and HbA1c level was estimated by chromatographic colorimetric method.

The quantitative sandwich enzyme immunoassay technique was used to determine serum ADMA level by using commercially available enzyme-linked immunosorbent assay (ELISA) kit (IBL international, Germany).

ADMA estimation was carried out on the following principle: ADMA is bound to the solid phase of the microtiter plate. ADMA in the samples is acylated and competes with solid phase bound ADMA for a fixed number of rabbit anti-ADMA antiserum binding sites. When the system is in equilibrium, free antigen and free antigen anti-antiserum complexes are removed by washing. The antibody bound to the solid phase ADMA is detected by anti-rabbit/peroxidase. The substrate tetramethylbenzidine (TMB)/peroxidase reaction is monitored at 450nm. The amount of antibody bound to the solid phase ADMA is inversely proportional to ADMA concentration of the sample.

SPSS15 was used for data analysis. P<0.05 was considered significant. Pearson's correlation co-efficient (r) was used to determine relationship of serum ADMA with other parameters.

### Results

Of the 150 subjects, there were 75(50%) each in Group A and Group B. The mean age was 50.8±8.0 years in Group A and 53.4±5.2 years in Group B (Table-1).

Group B patients had significantly higher mean levels of serum ADMA (03.9±1.5) than Group A (0.6±0.2), (p<0.0001). FBG showed significant increase (p<0.0001) in Group B (242.09±70.7) compared to Group A (96.41±18.4). Mean HbA1c showed significant difference (p<0.002) in

**Table-1:** Demographic and clinical/general parameters of the study groups.

General Parameters	Group A Normal	Group B (Diabetic with CAD)
Age in years	50.8±8.0	53.4±5.2
Systolic blood pressure (mmHg)	119.0±10.8	154.4±21.6
Diastolic blood pressure (mmHg)	81.1±9.1	92.2±10.2
Body Mass Index (Kg/m <sup>2</sup> )	25.9±3.0	32.4±3.0

CAD: Coronary artery disease.

**Table-2:** Biochemical parameters of the study group.

Bio Chemical Parameters	Group A Normal	Group B (Diabetic with CAD)
Asymmetric dimethyl arginine (μmol/L)	0.6±0.2	03.9±1.5
Glycosylated hemoglobin (%)	04.9±1.48	14.9±29.0
Total cholesterol (mg/dL)	154.3±22.8	307.4±160.1
Triglyceride (mg/dL)	118.0±55.0	284.3±150.2
Low density lipoprotein (mg/dL)	141.8±36.3	193.8±50.7
High density lipoprotein (mg/dL)	45.8±10.7	32.0±07
Fasting Blood Sugar (mg/dL)	99.7±18.40	196.4±98.5

**Table-3:** Correlation of serum ADMA with biochemical parameters.

Correlation	Group A (control)		Group B (Type 2 DM + CAD)	
	r	p	R	P
Age	0.060	NS	0.178	NS
SBP	0.178	NS	0.672	< 0.05
DBP	0.118	NS	0.785	< 0.05
BMI	0.344	< 0.05	0.383	< 0.05
FBG	0.204	NS	0.633	< 0.05
HbA1c	0.312	< 0.05	0.545	< 0.05
TC	0.523	< 0.05	0.392	< 0.05
TG	0.439	< 0.05	0.496	< 0.05
HDL-C	0.090	NS	0.065	NS
LDL-C	0.360	< 0.05	0.491	< 0.05

Correlation is significant at the 0.05 level.

NS: Non significant.

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

BMI: Body mass index

FBG: Fasting blood glucose

HbA1c: Glycated haemoglobin

TC: Total cholesterol

TG: Triglycerides

HDL-C: High-density lipoprotein cholesterol

LDL-C: Low-density lipoprotein cholesterol.

Group B ( $12.45 \pm 11.6$ ) was compared to the controls ( $4.9 \pm 1.48$ ). Lipid profile was significantly deranged in Group B with higher TC levels ( $307.4 \pm 160.3$  vs  $154.3 \pm 22.8$ ;  $p=0.000$ ) and significantly lower HDL-C levels ( $32.0 \pm 07$  vs  $45.8 \pm 10.7$ ;  $p=0.0002$ ) than Group A. Serum TG level was significantly higher in Group B ( $284.3 \pm 150.2$  vs  $118.0 \pm 55.0$ ;  $p<0.05$ ). LDL-C in Group B was significantly increased ( $193.8 \pm 50.7$  vs  $141.8 \pm 36.3$ ;  $p<0.05$ ) (Table-2).

A significant and positive correlation was observed between serum ADMA level and FBG ( $r=0.633$ ;  $p=0.001$ ), HbA1c ( $r=0.545$ ;  $p=0.001$ ), TG level ( $r=0.496$ ;  $p=0.001$ ) and LDL-C ( $r=0.491$ ;  $p=0.001$ ) whereas weak but significant correlation was found between ADMA and TC ( $r=0.392$ ;  $p=0.010$ ). In Group A, a highly significant positive correlation was observed between serum ADMA with TC ( $r=0.523$ ;  $p=0.0001$ ) and TG level ( $r=0.439$ ;  $p=0.0001$ ) whereas a significant correlation existed between serum ADMA with HbA1c ( $r=0.312$ ;  $p=0.009$ ) and LDL-C level ( $0.360$ ;  $p=0.002$ ) (Table-3).

## Discussion

CAD is caused by hardening and thickening of wall of blood vessels along with fatty deposits due to high glucose in T2DM.<sup>11,13</sup> One study<sup>15</sup> has also discussed that diabetics are at increased risk of atherosclerosis and it clinically manifests as CAD.

This study discusses clinical and biochemical parameters

(age, gender, BP, BMI, serum ADMA, TGs, TC, FBG, etc.) in T2DM patients which ultimately result in CAD. A similar study was undertaken earlier.<sup>16</sup>

In our study the mean age of majority of patients in Group B was 44-55 years, similar to earlier findings.<sup>17</sup> Similarly, Haffner et al<sup>18</sup> and Turner<sup>19</sup> have also shown that patients above 40 and 50 years age have twofold increased risk for CAD in T2DM.

According to Rita,<sup>20</sup> CAD in the absence of DM was lower among women but in T2DM-with-CAD group the ratio was found equal among both genders. Similarly, the study of Nesto and his colleagues<sup>21</sup> showed that both men and women are equally prone to develop CAD in T2DM. This is in contradiction with this present study as a majority of Group B patients were males. This shows that our male population is more prone to acquire all risk factors which will easily end in CAD.

BMI among Group B patients was more than controls with significant statistical differences ( $p<0.0002$ ). This shows that T2DM patients with increased BMI have developed CAD. All these analysis corresponds well with the study of Romero and his colleagues.<sup>22</sup>

Group B had the highest mean SBP and DBP than Group A ( $p<0.0001$ ). It has been well documented in the study of Ravi et al.<sup>23</sup> that the prevalence of HT is higher among diabetics with CAD. According to Gordon,<sup>24</sup> every 10mmHg increase of SBP in T2DM increases the risk of CAD by 15%.

Diabetics with CAD had significantly higher serum ADMA concentration than controls which shows its role in promoting cardiac disease. Peter<sup>25</sup> revealed ADMA as the potential risk factor glucose and TG were significantly higher in T2DM patients. In another study conducted by Abbasi et al (2001) showed that the levels of ADMA, plasma glucose and triglycerides were significantly higher in patients with type 2 diabetes mellitus.<sup>26</sup>

Evidence and association of CAD with hyperglycaemia (FBG, HbA1c) in T2DM came from McGill et al,<sup>27</sup> similar to the findings of this study.

All these predisposes to hardening and thickening of walls of blood vessels along with fatty deposits.

Wilhelmen et al.<sup>28</sup> have discussed the specific feature of dyslipidemia in patients of T2DM. He showed altered LDL, low HDL, raised TGs and TC. Mohan and Deepa<sup>24</sup> also analysed the association of increased LDL with increased age as the main risk factors for CAD in T2DM patients.

The study also showed raised TC and TGs levels ( $p=0.0001$ )

and decreased HDL levels ( $p=0.0002$ ) in the diseased group than the normal group.

### Conclusion

All clinical and biochemical parameters were found raised among diabetic patients with CAD. A significant positive correlation was seen between raised serum ADMA level and other biochemical parameters, except HDL-C in such patients.

**Disclosure:** No.

**Conflict of Interest:** No.

**Funding Sources:** No.

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