ORIGINAL ARTICLE

ROLE OF ASYMMETRICAL DIMETHYL ARGININE IN CARDIOVASCULAR DIABETOLOGY

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ABSTRACT

Objective: To determine the serum ADMA level and association of different biochemical parameters with ADMA in diabetes mellitus patients with and without coronary artery disease and normal healthy individuals.

Material and Methods: A total of 210 patients ,out of which 70 type 2 diabetics without coronary artery disease in group B and 70 type 2 diabetics with CAD in group C are included. 70 normal control individuals in group A were also enrolled in the study. Blood was obtained from each participant for biochemical analysis of fasting blood sugar (FBS), glycated hemoglobin (HbA1c), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG) and asymmetrical dimethyl arginine (ADMA).

Results: An elevated ADMA was noted in diabetic subjects with and without coronary artery disease than the healthy control group (03.9±1.5µmol/L and 02.0±0.6 Vs. 0.6±0.2 µmol/L). Similarly different parameters like FBS, HbA1c, HDL, LDL-C Triglyceride were found to be elevated in groupB and group C as compared to group A.

Conclusion: All biochemical parameters are found to be increased in both the diseased groups i.e type 2 diabetes mellitus without CAD and type 2 diabetes mellitus with CAD as compared to the normal healthy individuals.

Key Words: Asymmetric dimethylarginine (ADMA),Type 2 diabetes mellitus,Coronary artery disease, Glycosylated hemoglobin, Lipid profile.

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INTRODUCTION

Asymmetric dimethylarginine the formation of ADMA as well as NG-Mono methyl arginine (L-NMMA) requires the enzyme Arginine methyltransferase type 1 (PRMT 1) which takes part in the methylation of arginine residues, commonly occurring inside the nuclues and involved in the processing and transcription of RNA. ADMA is formed when these proteins are hydrolysed.[1,2] About 20% of ADMA is excreted through kidneys while the remaining 80% is degraded by an enzymedimethylarginine dimethylaminohydrolase[3,4]. To be transported inside the endothelial cell, there is competition between these methyl arginines and arginine which is a normal substrate for nitric oxide synthase, as a result decrease in NO synthesis occurs if there is increased circulating levels of ADMA[5,6,7]. The prevalence of Diabetes Mellitus is increasing day by day and is a common group of metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fats and protein metabolism which results from absolute or relative deficiency of insulin or its action or both[5]. Because the hyperglycemia in Diabetes Mellitus is responsible for increased tissue breakdown leading to increased serum ADMA level, secondly hyperglycemia impairs the function of DDAH that causes 80% of ADMA elimination from the body, increasing circulating ADMA[8]. ADMA is considered an indicator of endothelial dysfunction as it causes reduced NO synthesis levels[1,9]. Mortality due to myocardial infarction or ischemic heart disease account for a large fraction in diabetic patients[10,11]. Patients who suffer from diabetes mellitus are at high risk of vascular complications, therefore DM is thought to be a major risk factor for developing cardiovascular disease[12]. Plasma glucose concentration in the diabetic patients is directly related to the ADMA concentrations[13,14] therefore a strict plasma glucose control among diabetic type 2 patients may have antiatherogenic effects through reduced ADMA level[15]. Insulin resistance patients had a significant level of elevated plasma ADMA concentrations which provide evidence that ADMA may act as a risk

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factor for the cardiovascular diseases[16,18]. The current study was undertaken for evaluating association of ADMA with different biochemical parameters inT2DM with and without CAD and compare these to normal controls. Also to define the role of ADMA in the development of CAD.

MATERIAL AND METHODS

An analytical/ cross sectional study was conducted from October 2010 to 2011 to view the difference of plasma ADMA level among patients having diabetes mellitus with and without coronary artery disease attending the Outpatient Medical and Cardiology Departments of Khyber Teaching Hospital and Hayatabad Medical Complex, Peshawar. The study was approved by the Ethical Review Committee of Khyber Medical College, Peshawar.

The study population of 210 were divided into Group A - with people having no diabetes mellitus, heart diseases or any other disease,Group B - with patients suffering from type 2 diabetes mellitus and Group C –with patients having type 2 diabetes mellitus with coronary artery disease. The study participants included had age range of 35-65 years. Patients already diagnosed and having type 2 diabetes mellitus for the last three years were included. Patients with type 2 diabetes mellitus and having had a myocardial infarction in the last seven days were included in the study group C. Patient using lipid lowering therapy or rennin angiotensin system (RAS) inhibitors were not included in the study. Patient having febrile illness, infection, inflammatory diseases, gastrointestinal, liver, thyroid and kidney diseases etc. Were excluded from the study.

After obtaining informed consent, demographic details, complete clinical history and relevant physical examinations were done and all the information was recorded in a pre-prepared structured data collection proforma.

Blood samples were collected by aseptic technique after 12 hours overnight fasting. The blood was centrifuged at 3000rpm for 5min and serum was separated. The serum was collected in eppindorf tubes which were appropriately labeled and stored at -20°C for further analysis of different parameters like fasting blood glucose, triglycerides,total cholesterol, HDL-C and ADMA. A portion of blood sample was put in EDTA bottle for estimation of HbA1c level. Blood glucose (fasting), total cholesterol level, total triglyceride and HDL-cholestrol were analyzed by the enzymatic colorimetric method. LDL was calculated using Friedewald formula[19], and HbA1c level was estimated by chromatographic colorimetric method. Serum ADMA level was measured using commercially available enzyme-linked immunnosorbent assay (ELISA) kit.

SPSS version 15 was used to record, store, and assess the collected data. The results were summarized by using both descriptive and inferential statistics. A P-value of < 0.05 was considered as statistically significant. Pearson's correlation coefficient was used to determine the relationship of serum ADMA with other parameters.

RESULTS

As shown in Table 1 the age distribution of the participants ranged from 35 to 65 years with mean of

Table 1: General parameters of the study groups

General parameters	Group A (normal)	Group B (diabetics)	Group C (diabetics without CAD)
Age in years	50.8±8.0	54.4±5.2	56.0±4.1
Systolic blood pressure (mm Hg)	119.0 ±10.8	154.4±21.6	151.6±25.7
Diastolic blood pressure (mm Hg)	81.1±9.1	92.2±10.2	92.6±2.0
Body Mass Index (Kg/m2)	30.9±3	28.4±3	34.9±31.0

Table 2: Biochemical parameters of the study groups

Biochemical parameters	Group A (normal)	Group B (Diabetics)	Group C (Diabetics with CAD)
Fasting blood sugar(mg/dl)	99.7±18.4	170.5±60.7	196.4±98.5
Glycosylated Hemoglobin(%)	04.9±1.48	07.4±2.8	14.9±29.0
Total Cholesterol (mg/dl)	154.3±22.8	278.5±124.0	307.4±160.1
Triglyceride(mg/dl)	118.0±55.0	276.2±154.0	284.3±150.2
Low density lipoprotein(mg/dl)	141.8±36.3	167.6±38.7	193.8±50.7
High density lipoprotein (mg/dl)	45.8±10.7	38.5±9.3	32.0±07
Asymmetrical dimethyl arginine(µmol/L)	0.6±0.2	02.0±0.6	03.9±1.5

Table 3: Correlation of asymmetric dimethl arginine with different biomedical parameters

Biomedical Indicators	Group A		Group B		Group C	
	r vlue	p value	r vlue	p value	r vlue	p value
Age in year	0.060	NS	0.143	NS	0.178	NS
Systolic Blood Pressure	0.178	NS	0.051	NS	0.672	<0.05
Diastolic Blood Pressure	0.118	NS	0.055	NS	0.785	<0.05
Body Mass index	0.344	<0.05	0.073	NS	0.383	<0.05
Fasting blood Sugar	0.204	NS	0.743	<0.05	0.633	<0.05
Glycosylated Hemoglobin	0.312	<0.05	0.682	<0.05	0.545	<0.05
Cholesterol level	0.523	<0.05	0.204	NS	0.392	<0.05
Low density lipoprotein	0.360	<0.05	0.508	<0.05	0.491	<0.05
Triglyceride level	0.439	<0.05	0.084	NS	0.496	<0.05
High density lipoprotein	0.090	NS	0.288	<0.05	0.065	<0.05

NS: Non significant. Correlation is significant at the 0.05 level.

53.73 ± 6.436 SD. Further detailed categorization of age indicates that 97 (46.2%) subjects were from 46 to 55 years of age group followed by 85 (40.5%) in 56 to 65 years, while those aged <45 years of age accounted for the smallest proportion 28 (13.3%). As compared to the normal, the diseased groups (diabetic with and without coronary artery disease) had high blood pressure (systolic), body mass index (BMI). Our study finding revealed that the mean systolic blood pressure among diabetic without and with coronary artery disease (154.4±21.6, 151.6±25.7) was significantly raised than the normal group (119.0±10.8) with a P <0.0001. Similarly, body mass index (BMI) among these groups had also significant statistical differences (p < 0.0002) among the three groups, however; the cardiac patients with diabetes presented with the highest BMI.

Table 2 shows the biochemical parameters of the study groups, as compared to the normal, the diseased groups (diabetic with and without coronary artery disease fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and asymmetric dimethyl arginine (ADMA) levels. However no significant difference was found between the three comparative groups with respect to age.

The diabetic patients with coronary heart disease had significantly higher serum ADMA concentration than simple diabetics and normal healthy subjects (03.9±1.5µmol/L vs. 02.0±0.6 and 0.6±0.2 µmol/L) p <0.0001. Fasting blood glucose showed significant increase (p<0.0001) in diabetic patients without and with cardiovascular disease (170.5±60.7 and 196.4±98.5) as compared to healthy normal control (99.7±18.4). In the same way, the mean glycosylated hemoglobin (HbA1c) showed significant difference (p<0.002) in diabetic patients with and without cardiovascular disease (07.4±2 and 14.9±29.0) as compared to the healthy control group (04.9±1.48) . The FBG and HbA1c were highest in cardiac patients with diabetes mellitus. The results of lipid profile showed significantly raised total cholesterol and triglyceride levels (P value 0.0001) and decreased HDL levels (P value 0.0002) in the diseased groups than the control.

Table 3 shows the association of serum ADMA with the different biochemical parameters , A significant strong positive correlation was observed between serum ADMA level and FBS(r=0.743 ,p=0.001), HbA1c (r=0.682, p=0.001) and LDL-C (r=0.508, p=0.001) respectively. A significant correlation exists between serum ADMA and HDL-C (r=0.288 , p=0.016), whereas a nonsignificant weak correlation was found between ADMA and total cholesterol level (r =0.204, p =0.090) in group B while a significant and strong positive correlation was observed between serum ADMA level and FBS (r =0.633, p= 0.001), HbA1c (r=0.545, p=0.001), triglyceride level (r=0.496, p=0.001) and LDL –C (r=0.491, p=0.001), whereas a weak significant correlation was found between ADMA and total cholesterol (r=0.392, p=0.010) in group C as compared to normal group A.

DISCUSSION

Coronary artery disease has become a major cause of morbidity and mortality in patients with type II diabetes mellitus (T2DM)[20] in whom hyperglycemia causes disturbances in different organ systems and is affected by ADMA[67]. Hence control of hyperglycemia is important to manage T2DM[21]. The diabetics have poor prognosis leading to atherosclerosis and ischemic heart disease (diabetic cardiomyopathy) which seems to enhance myocardial dysfunction leading to an increased

frequency of heart failure. These patients again can have a lot of complications including high levels of plasma cholesterol, triacylglycerol, LDL and low levels of HDL. In addition, these patients also have a predominance of cellular abnormalities like formation of smaller, denser particles which in turn leads to the process of atherogenicity even in the presence of absolute concentration of HDL[22,23]. Our finding indicated that serum ADMA level was1high (03.9±1.5 µmol/L) among diabetics with CAD2 as to diabetics. 1.0±0.6 µmol/L) and normal (0.6±0.2 µmol/L) controls. This indicates that increased serum ADMA among T2DM patient with coronary artery disease is due to vascular damage in type 2 diabetic patients which results from high ADMA levels causing vascular diseases. These finding are in agreement with Maas et al (2003)[24]. They tested hyperglycemia's effect on ADMA level in laboratory and clearly explained the mechanism by which hyperglycemia cause shigh ADMA concentration. They explained this by linking the synthesis of ADMA by the enzyme arginine methyl transferase, increasing its concentration. As hyperglycemic condition induce soxidative stress which in turn regulates arginine methyltransferases and causing the impairment of serum ADMA level. This further leads to the endothelial dysfunction and leads to vascular complications and cardiac diseases[24,25]. Mahfouz et al. (2009) showed significant difference between FBS levels in diabetic patients with and without CAD than the normal control group, so was the concentration of ADMA among the groups[26]. Lin et al (2003), showed that ADMA is increased by hyperglycemia which causes impairment of DDAH activity causing endothelial dysfunction, aggravated by oxidative stress leading to CAD, seen in diabetics patients[6]. Abbasi et al (2001) reported a positive correlation of serum ADMA concentration with fasting blood glucose level[13].The findings of our study are also in accordance with the above groups and revealed a positive relationship between ADMA level and fasting blood glucose (r = 0.366).[1] A significant and positive association of serum ADMA level and HbA1c in normal control group A (r = 0. 312, p = 0. 009), Subjects with type 2 diabetes mellitus (3r =0. 682, p = 0. 001) type 2 diabetic with CAD, (r = 0. 545, p = 0.001). Devangelio et al (2007) conducted a study showing that ADMA2 level was significantly higher in diabetic patients and HbA1c and ADMA were directly correlated. They also found that9improving metabolism result in low ADMA level and controlled FBS and HbA1c[27]. In contrast Paiva et al (2003) showed that ADMA was inversely correlated with HbA1c and Xiong et al (2004) found that increased ADMA level had no significant correlation with diabetes mellitus duration, but documented a significant correlation with glycemic control[28,29]. An increase in total cholesterol, triacylglycerol and low-density lipoprotein (LDL) levels were found among diabetic patients as compared to normal healthy individuals. Furthermore the result also showed pronounced increase in the parameters in diabetic patients having evidence of coronary artery disease than the control group. Our results were similar to previous studies on diabetics by Gordon et al (2010) which stated that lipoprotein abnormalities enhance because of diabetes[30,31]. Yasuda et al (2006) conducted a study on type 2 diabetes mellitus with uncontrolled glycemia, of which some were conventionally treated and the rest were on intensively treated. They found that FBS, total cholesterol and LDL decreased significantly with intensive treatment[15]. Morton J et al (2013) showed that type 2 DM is usually associated with high TG and low HDLs levels as seen in our study. They showed that this arrangement was strongly linked4with a high risk of developing cardiovascular diseases[32,36].

CONCLUSION

Increased serum ADMA levels were observed in both the diseased groups i.e type 2 diabetes mellitus without CAD and type 2 diabetes mellitus with CAD as compared to the normal healthy individuals. Furthermore a significant positive correlation was seen between serum ADMA levels and fasting blood glucose, HbA1c, total cholesterol, HDL-c and triglyceride level in type 2 diabetic patients with and without CAD. . Furthermore, these findings suggest a strong role of serum ADMA level in coronary artery disease development and progression.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Tariq K: Main idea.

Khan MA: Critical review.

Khattak N: Data collection and bibliography.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.