CORRELATION OF SEX DIFFERENCE AND ASYMMETRIC DIMETHYL ARGININE WITH DIABETES MELLITUS AND CORONARY ARTERY DISEASE

Kalsoom Tariq[1], Bela Inayat[1], Amin Ul Haq[1], Gulnaz Begum[1], Muddasir Ahmad Khan[2], Nabila Sher[1]

ABSTRACT

OBJECTIVE: To determine the correlation of sex difference with diabetes and CAD and to find out association of serum **Asymmetric dimethyl arginine** with glycosylated hemoglobin in type 2 diabetic patients with and without coronary artery disease.

METHOD: This cross sectional study was conducted in Khyber Teaching Hospital and Hayatabad Medical Complex from October 2010 to October 2011 to evaluate ADMA in type 2 diabetes Mellitus patients with and without coronary artery disease. This comprised of three groups of 70 participants in each. Group A was normal individuals, B consisted of patients suffering from type2 diabetes mellitus, while C comprised of patients having type 2 diabetes mellitus with coronary artery disease.

RESULTS: An elevated ADMA was noted in group B and Group C (03.9±1.5µmol/L and 02.0±0.6) Group A (0.6±0.2 µmol/L). In group B, a significant strong positive linear relationship was seen between ADMA level and FBS (r = 0.743 , p =0.001) , HbA1c (r=0.682 , p = 0.001) , LDL (r = 0.508 , p = 0.001).Among group C strong correlation was noted between serum ADMA level and fasting blood glucose (r = 0.366, p = 0.0001), total cholesterol and triglyceride level (r = 0.392, p = 0.010; r = 0.496, p = 0.001) respectively. However, sex difference had no impact on ADMA & thus on diabetes or CAD.

Conclusion: In this study serum ADMA was elevated in type 2diabetic patients whether they had CAD or not. There was a significant correlation between ADMA and HbA1c.

Keywords: Asymmetrical dimethylarginine, Coronary artery disease, Type 2 diabetes mellitus, Glycosylated hemoglobin.

INTRODUCTION

Oxidative stress is found to be a contributing factor to the development of both micro and macro angiopathies[1] . Nitric oxide (NO) is a gas free radical and inorganic in nature. It acts as a biological messenger [2]. The diffusion of NO into the endothelial layer occurs, resulting in vascular dilation, playing a vital role in maintenance of vascular composition as well as its tone[3],[4]In diabetic patients, oxidative stress has been reported to be largely because of free radical formation in increase amount and a quick decrease in the defense by antioxidants [5].

1. Khyber Girls Medical College, Peshawar
2. Khyber Medical College, Peshawar

Address for Correspondence:
Dr. Nabila Sher
Associate Professor, Department of Biochemistry, Khyber Girls Medical College, Peshawar
dr.nabi65@gmail.com

Nitric oxide takes part in the insulin secretion through pathway of signal transduction and play important role in β- cell destruction in diabetic patients[6]. It acts as a culprit in the injury of β- cells in diabetes mellitus[6]. It has been predicted by World Health organization that incidence of Type II diabetes mellitus (T2DM) will increase up to 300 million by 2025 in the world [7]. This steady increase in the incidence of T2DM place a significant burden on the health care services because of its complex consequences on the other systems of the body hence needs early detection, treatment and prevention [8]. The mortality and morbidity due to chronology of types of diabetes results from complications of different organopathy i.e. diabetic neuropathy, retinopathy and nephropathy etc. such complications are consequences of metabolic disorder which leads to hyperglycemia [9],[10].

Endothelial dysfunction has a potential contribution to the pathogenesis of vascular diseases among patients with diabetes mellitus [11]. Atherosclerosis leading to vascular disease has been linked with a decrease bioavailability of nitric oxide in the body tissue [12] .Serum ADMA acts as an endogenous nitric

oxide synthase (NOS) inhibitor by competing with arginine for the active site. This leads to reduce NO generation thus aggravating the condition[3]. Cardiovascular problems are the main cause of mortality and morbidity approximately 17.3 million deaths per year, which is further estimated to rise >23.6 million by 2030 [13].Hyperglycemia impairs the enzyme, Dimethyl Arginine Dimethyl Amino Hydrolase (DDAH) which is responsible for the degradation of ADMA, thus leading to high concentration of ADMA in blood [14]. This high concentration of ADMA can reduce the transport of L-arginine inside the cells, causing a decrease in nitric oxide synthesis and endothelial dysfunction [15]. Being an inflammatory marker, ADMA is increased in the plasma of humans in different inflammatory conditions like cardiac diseases, diabetes, atherosclerosis, hypercholesterolemia, hypertension and chronic renal failure [16].

Potential mechanism for the sex differences in the impact of T2DM on coronary artery disease (CAD) risk includes biological & physical factors plus disparities in disease management between sexes [17]. Women develop diabetes at a higher BMI so may experience prolonged exposure to CAD risk factors prior toT2DM diagnosis compared to men. Similarly gender differences in diet and physical activity may be one of the reasons as these maybe lower in females than men [18].Research is being carried out throughout the world to explore the role of ADMA in development of complications in type 2 diabetes mellitus.

METHODOLOGY: This cross sectional study (enrollment no.06/Bio/KMC) was conducted to assess the correlation of sex differences and also ADMA with T2DM and coronary artery disease. Ethical approval was obtained from the Ethical Review Committee of Khyber Medical College(94-AMC-274). This one year study (October2010- October 2011) consisted of three groups, each of 70 participants. The sample size was based on specific absolute precision, using the formula $n= z^2 1-\alpha\ P\ (1-P) / d^2$

Where, Prevalence (P = 0.18), Confidence Level (% 1-α = 95% , Absolute precision / Margin of error(d = 0.05). The sampling technique used was 'simple random sampling method'. Normal participants taken as control were included in group A. Patients having only T2DM (for the last three years) were in group B while group C comprised of patients having T2DM and CAD (having had a myocardial infarction in the last seven days). People having diseases of the gastrointestinal tract, liver, thyroid or any inflammatory disease and febrile illness were excluded from this study. Also those who were taking lipid lowering drugs or rennin angiotensin system (RAS) inhibitors were not included in the study.

Following informed consent, demographic information, a thorough clinical history, and pertinent physical tests were completed, and all information was documented in a pre-prepared structured data collecting proforma. Following a 12-hour overnight fast, blood samples were taken aseptically. The serum was extracted after blood was centrifuged at 3000rpm for 5 minutes. The serum was collected in eppindorf tubes that were correctly labeled and kept at -20°C for future investigation of various parameters.

Enzymatic colorimetric method was used for the estimation of total cholesterol level, total triglyceride, HDL-cholesterol and blood glucose. Friedewald formula was used for calculating LDL [19] and the chromatographic colorimetric technique was used to calculate HbA1c levels. All laboratory studies were carried out at Khyber Medical College's Research Laboratory, Biochemistry Department, Peshawar.

SPSS version 15 was used for the assessment of data. Anderson Darling test was used to determine normality of the data. Pearson's correlation was used for comparison among the groups and P value of >0.05 was considered statistically significant.

Operational definitions:

Diabetes mellitus: Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves.

Cardiovascular disease: is a class of diseases that involve the heart or blood vessels. CVD includes coronary artery diseases (CAD) such as angina and myocardial infarction (commonly known as a heart attack).

RESULTS: Out of 210 participants 100 (47.6%) were males.The overall gender ratio was alike in diabetic patient having cardiac disease group and normal control group i.e 37 (52.9%) male 33 (47.1%) females. However

the female ratio in diabetic group was a little high 44(62.9%) as compared to the rest of the two groups (Table 1).

Table 1: Gender distribution of the study groups

	Gender	
Study Groups	**Male**	**Female**
Diabetic	26 (37.1%)	44(62.9%)
Diabetic Cardiac	37 (52.9%)	33 (47.1%)
Normal	37 (52.9%)	33 (47.1%)
Total	**100 (47.6%)**	**110 (52.4%)**

Table 2 shows that the 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.

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. 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) $p <0.0001$.

However no significant difference was found between the three comparative groups with respect to age and gender.

Table 2: laboratory findings of the study groups

	Group A **Normal**	**Group B** **(Diabetic)**	**Group C** **(Diabetic with CAD)**
Age in year	50.8±8.0	54.4±5.2	56.0±4.41
Body Mass Index (Kg/m²)	30.9±3.0	28.4±3.0	34.9 ±31.0
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
Asymmetric dimethyl arginine (µmol/L)	0.6±0.2	02.0±0.6	03.9±1.5

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 non-significant weak correlation was found between ADMA and total cholesterol level (r =0.204 ,p =0.090) **(Table 3).**

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)**(Table 3).**

Table 3: Correlation of asymmetric dimethylarginine (ADMA) with different parameters among the groups

Biomedical Indicators	**Group A**		**Group B**		**Group C**	
	r- value	**P –Value**	**r- value**	**P –Value**	**r- value**	**P –Value**
Age (yr.)	0.060	0.621	0.143	0.238	0.178	0.140
Body Mass Index(Kg/m²)	0.344	0.004	0.073	0.549	0.383	<0.001***
Fasting Blood Sugar (mg/dL)	0.204	0.090	0.743	0.001	0.633	<0.001***
Glycosylated hemoglobin (%)	0.312	0.009	0.682	0.001	0.545	<0.001***
Cholesterol level (mg/dL)	0.523	0.0001	0.204	0.090	0.392	<0.010**
Triglyceride level (mg/dL)	0.439	0.0001	0.508	0.001	0.496	<0.001**
High-density lipoprotein (mg/dL)	0.090	0.458	0.084	0.490	0.065	0.594
Low-density lipoprotein (mg/dL)	0.360	0.002	0.288	0.016	0.491	<0.001***

Significant difference (*P<0.05, **P<0.01, ***P<0.001)

Figure 1 shows association between serum ADMA and HbA1c in type 2 diabetic patients. It shows a highly significant and strong positive correlation between ADMA and HbA1c.

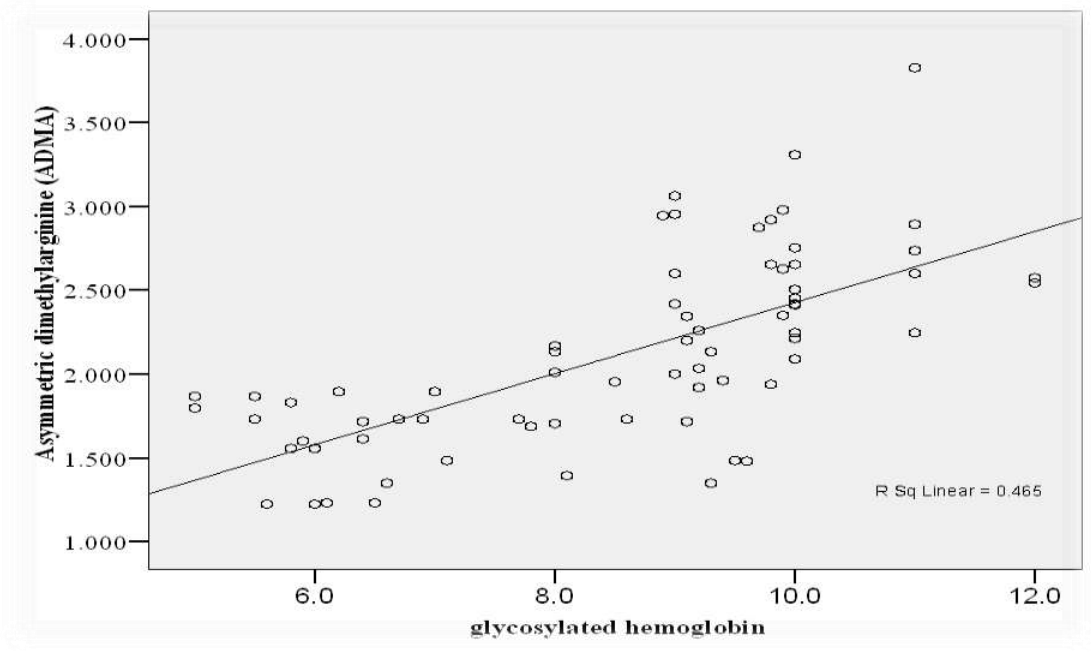

Figure 1: Correlation between serum ADMA and HbA1c (%) in type 2 diabetic patients (r = 0.682, p =0.001).

Figure2 shows association between serum ADMA and HbA1c in type 2 diabetic patients with coronary artery disease. It shows a highly significant positive correlation between ADMA and HbA1c.

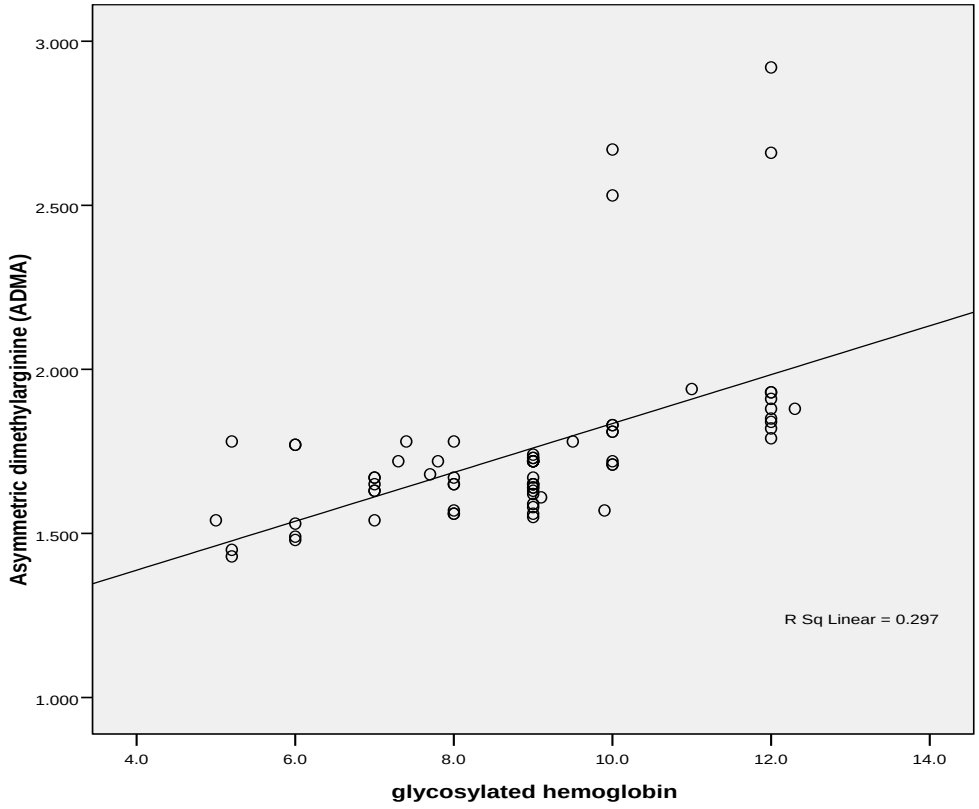

Figure 2: Correlation between serum ADMA and HbA1c in type 2 diabetic patients with CAD (r = 0.545, p =0.001)

DISCUSSION

Coronary artery disease has become a major cause of morbidity and mortality in patients with T2DM [20], in whom hyperglycemia is one of the main metabolic abnormalities and is affected by ADMA [21]. The patients with DM have poor prognosis leading to atherosclerosis and ischemic heart disease [22] .

This study showed that age and sex differences had no impact on the causation of T2DM or CAD. These findings are in accordance with the study done by Yahagi *et al.*, who documented that men and women share the majority of the underlying systemic risk factors for coronary artery disease. However, the influence of numerous risk factors differs across men and women, with smoking being a greater danger in women than in men, particularly among younger women [23]. In contrast various studies have shown that gender and age do play a role in the causation and progress of T2DM and CAD[17],[18] .

Our finding indicated that serum ADMA level was high (03.9±1.5 µmol/L) among diabetic patients with CAD as compared to diabetic patients without CAD (02.0±0.6 µmol/L) and normal (0.6±0.2 µmol/L) subjects. This indicates that high plasma concentration of ADMA among T2DM patient with coronary artery disease is due to vascular damage in type 2 diabetic patients which results in the endothelial dysfunction associated with increased ADMA concentrations. These finding are in agreement with Maas *et al*., they tested the effect of hyperglycemia on ADMA level in laboratory and clearly explained the mechanism for the increase in ADMA concentrations in hyperglycemic media. According to them, increase in ADMA concentrations is directly linked with the enzyme arginine methyl transferase, which synthesizes ADMA. As hyperglycemic condition induces oxidative stress which in turn regulates the expression of arginine methyl transferases and finally leads to the impairment of ADMA concentrations in the body. This further leads to the endothelial dysfunction and leads to vascular complications and cardiac diseases [24].

Ghosh *et al*. found significantly higher FBG levels in patients with type 2 diabetes mellitus with and without CAD as compared to the normal control group and so was the concentration of ADMA among the groups [25]. Zadhoush *et al.* reported a positive correlation of serum ADMA concentration with fasting blood glucose level [26].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).

We found a significant positive correlation between serum ADMA level and HbA1c among normal healthy individuals (r = 0.312, p = 0.009) , patients with type 2 diabetes mellitus (r =0.682 , p = 0.001) type 2 diabetic with CAD, (r = 0.545, p = 0.001). Mc Nair *et al*. conducted a study showing that ADMA level was significantly higher in diabetic patients and HbA1c and ADMA were directly correlated .They also found that improvement in metabolic control resulted in a decrease in ADMA level associated with a decrease in FBS and HbA1c [27].In contrast in a study by Takaya found that ADMA was inversely correlated with HbA1c [28 29].

An increase in serum 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 as compared to the normal group. These results are similar to previous studies done on diabetics by Gordon *et al* [30]. Morton J *et al.*explained that type 2 DM is usually associated with low plasma level of HDL and is often accompanied by elevated TG levels as seen in our study .They showed that this arrangement was strongly linked with an increase in risk of developing coronary artery diseases [31]. In the present study the serum HDL levels were low (38.5±9.3 mg/ dl) among patients with type 2 diabetes mellitus as compared to diabetics with coronary artery disease (32±07mg/dl) and normal healthy subjects (45.89±10.1 mg/dl)

Sibel *et al.* showed the effect of statin therapy on serum ADMA level , they found that increase LDL-cholesterol levels were associated with increase serum ADMA level and this elevated ADMA concentration is associated with increased risk of cardiovascular disease[32] .Our study also showed increase serum level of LDL in diabetic patients with CAD (193.8 $\pm$ 50.7) and in type 2 diabetic patients (167.6 $\pm$ 38.7) as compare to the normal control group (141.8 $\pm$ 36).

CONCLUSION

In conclusion, our study reveals increased serum ADMA levels in both the diseased groups i.e type 2 diabetes mellitus and type 2 diabetes mellitus with CAD as compared to the normal healthy individuals. Additionally, in type 2 diabetes patients with and without CAD, there was a strong positive connection between serum ADMA levels and fasting blood glucose, HbA1c, total cholesterol, HDL-c, and triglyceride levels.

Conflict of interest: none

Source of funding: none

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