ENDOTHELIAL DYSFUNCTION AND DERANGED LEVEL OF ASYMMETRIC DIMETHYL ARGININE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Objective: To determine the serum Asymmetric dimethyl arginine (ADMA) level, association of various biochemical parameters with plasma ADMA level in type 2 diabetic patients and also to compare with normal healthy individuals.

Methodology: Analytical cross sectional study was conducted at Medical and Cardiology Departments of Khyber Teaching Hospital (KTH) and Hayatabad Medical Complex (HMC), Peshawar for a period of one year. A total of 140 participants with age range of 35-65 years, were divided into two groups. Blood was obtained from each participant for biochemical analysis. All data obtained was entered in SPSS version 15. A P-value of < 0.05 was considered as statistically significant.

Results: An elevated ADMA was noted group B. In group A significant positive correlation was observed between serum ADMA with TC and TG level and a non-significant correlation was observed between ADMA and HDL level. In case of group B a significant strong positive linear relationship was seen between ADMA level and different parameters like FBG, HbA1c, LDL and no significant correlation was seen between ADMA with TG and TC.

Conclusion: Our study reveals increased serum ADMA levels in the patients suffering from type 2 diabetes mellitus without CAD when compared with the control group. Also a significant positive correlation was seen between serum ADMA level and FBS, HbA1c, TC, HDL-C and TG levels among the subjects of group B.

Key words: Type 2 diabetes, Coronary artery disease (CAD), ADMA, HbA1c, TC, Lipid profile, TG.

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INTRODUCTION

Asymmetric dimethyl arginine (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[1,2]. It is eliminated from the body by renal excretion (20%) and also metabolized (80%) by hydrolytic degradation to citrulline and dimethyl amine by the enzymatic action of dimethyl arginine dimethyla minohydrolase (DDAH)[3,4]. 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[5,6,7].

Diabetes mellitus is a chronic metabolic disease characterized by insulin deficiency (type 1) or insulin resistance (type 2) and a high blood glucose concentration (random glucose > 180 mg/dL, 2 hours after a meal or fasting plasma glucose > 125 mg/dl)[5]. Hyperglycemia in type 2 diabetic patients impairs an enzyme, DDAH which is responsible for the degradation of ADMA, thus leading to high concentration of ADMA in blood[8].

Type 2 diabetes has two major complications. Micro-vascular complications include retinopathy (damage to retina), neuropathy (damage to nerves) and nephropathy (damage to kidneys)[9,10]. The macro-vascular complications include hypertension, deranged lipid metabolism and arteriosclerosis leading to cardiac artery disease and cerebrovascular stroke. There is 2 to 4 times increase in the incidence of coronary artery disease (CAD) and 10 times increase in peripheral vascular disease resulting in atherosclerosis, diabetic retinopathy and nephropathy[11,13]. Cardiovascular problems are the main cause of mortality and morbidity for the 135 million individuals internationally with type 2 diabetes mellitus[12,13].

Looking at the important aspect of pathophysiology of ADMA in type 2 diabetes, the present study was undertaken to evaluate the association of ADMA and type 2 diabetes at Medical and Cardiology Unit of Khyber Teaching Hospital (KTH) and Hayatabad Medical Complex (HMC), Khyber Pukhtunkhwa (KPK).and to compare it to normal individuals. The study was conducted to find any possible relationship between serum ADMA level and other parameters in type 2 diabetic patients.

METHODOLOGY

A cross sectional study was conducted from October 2010 to October 2011 to view the difference of plasma ADMA level among patients having type 2 diabetes mellitus without CAD attending the Outpatient Medical and Cardiology Departments of KTH and HMC Peshawar, KPK.

The study was approved by the Ethical Review Committee of Khyber Medical College, Peshawar.

Total of 140 subjects were divided into two equal groups

- Group A - Included normal healthy individuals.
- Group B - Included patients suffering from type 2 diabetes mellitus.

The age range of participants was 35-65 years. People having no diabetes mellitus, heart diseases or any other disease were included in group A. Patients (group B) already diagnosed with type 2 diabetes mellitus for the last three years were included in the study. Patients using lipid lowering therapy or rennin angiotensin system (RAS) inhibitors and those with febrile illness, infection, inflammatory diseases, gastro-intestinal, liver, thyroid and kidney diseases were excluded from the study.

After obtaining informed consent and demographic details, complete clinical history was taken and relevant physical examination was done. 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 3000 rpm for 5 min 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. The samples were analyzed for fasting blood glucose, triglyceride, total cholesterol, HDL-C and ADMA levels.

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-cholesterol were analyzed by the enzymatic colorimetric method. LDL was calculated using Friedewald formula, and HbA1c level was estimated by chromatographic colorimetric method. Serum ADMA level was measured using commercially available enzyme-linked immunnosorbent assay (ELISA) kit.

All lab investigations were done at Research Laboratory, Biochemistry Department, Khyber Medical College, Peshawar, Khyber Pakhtunkhwa.

SPSS version 15 was used to record, store, and assess the collected data. The results were summarized by using both descriptive and inferential statistics. Descriptive statistics was used to calculate percentages and graphs, for categorical variables while means + SD was calculated for continuous variables like age, HbA1c and ADMA etc. Inferential statistic was also used to observe mean scores and was compared using student's t test, to observe the difference among the groups. A P-value of < 0.05 was considered as statistically significant.

RESULTS

The age distribution is demonstrated in table 1 indicating that the overall gender ratio was M: F 1.5:1, (n= 37, (52.9%) male and (n=33, (47.1%) females in group A. However the M: F 1:1.75 ratio in group B was slightly more (n=44, 62.9%) than group A.

Table 2 shows various demographic and laboratory parameters in different groups of the study participants. As compared to the normal control group A participants, group B patients had significantly high fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), low density lipoprotein-c (LDL-c) and asymmetric dimethyl arginine (ADMA) levels. Whereas the levels of HDL-c were significantly lower in group B patients when compared with group A individuals.

The association between serum ADMA level with hyperglycemia and lipid profile in group A is shown in table 3. A highly significant positive correlation was observed between serum ADMA with total cholesterol ($r = 0.523$, $p = 0.0001$) and triglyceride level ($r = 0.439$, $p = 0.0001$), whereas a significant correlation exists between ADMA with HbA1c ($r = 0.312$, $p = 0.009$), LDL-C level ($r = 0.360$, $p = 0.002$) and FBS ($r = 0.204$, $p = 0.009$). A non- significant correlation was observed between ADMA and HDL-C ($r = 0.090$, $p= 0.458$).

The association of serum ADMA with hyperglycemia and lipid profile among group B patients is shown in table 4. 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 ($r = 0.204$, $p = 0.090$).

Mean differences in various parameters were estimated using student's t test analysis. The two groups had significant differences in HbA1c, FBS and ADMA levels. We have graphically reported the plasma mean values of these different parameters in normal subjects and type 2 diabetes patients. The scattered plots (Figs. 1, 2, 3 and 4) indicate increased serum concentration of these parameters in patients of group B as compared to group A.

DISCUSSION

Diabetes mellitus is an emerging epidemic that has a potential burden on the health care services all over the world. Diabetes mellitus type 2 is the only effective disease that has been classified as an epidemic. The World Health Organization has predicted that incidence of type 2 diabetes will increase up to 300 million by 2025 in the world. Hyperglycemic state in type 2 diabetes is one of the main metabolic abnormalities and through a number of complex mechanisms leads to endothelial dysfunction, one of which is elevated serum ADMA levels[15,16,17].

Our study showed high serum ADMA levels high (03.9±1.5 µmol/L) among type 2 diabetic patients, (02.0±0.6 µmol/L) when compared to normal (0.6±0.2 µmol/L) subjects. This high plasma concentration of ADMA may lead to vascular damage and endothelial dysfunction. Maas et al[18] observed similar findings. According to them; increase in ADMA concentrations is directly linked with the enzyme arginine methyl transferase, which synthesizes ADMA. As hyperglycemic condition induces 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. Mahfouz et al[19] found significant higher FBG and ADMA levels in patients with type 2 diabetes mellitus. Lin and his colleagues,[20] stated that hyperglycemia increases ADMA by impairing DDAH activity in vascular smooth muscles and the endothelium,

Table 1: Gender distribution of the study groups

Study Groups	Gender	
	Male	Female
Group A(Normal)	37 (52.9%)	33 (47.1%)
Group B (Diabetic)	26 (37.1%)	44(62.9%)
Total	63 (45%)	77 (55%)

Table 2: Demographic and Laboratory Findings of the Study Groups

	Group A (Normal)	Group B (Diabetic)
Age in year	50.8±8.0	54.4±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^2)	30.9±3.0	28.4±3.0
Fasting Blood Sugar (mg/dL)	99.7±18.4	170.5±60.7 **
Glycosylated hemoglobin (%)	04.9±1.48	07.4±2.8 *
Total cholesterol (mg/dL)	154.3±22.8	278.5±124.0**
Triglyceride (mg/dL)	118.0±55.0	276.2±154.0**
Low density lipoprotein (mg/dL)	141.8±36.3	167.6±38.7
High density lipoprotein (mg/dL)	45.8±10.7	38.5±9.3*
Asymmetric dimethyl arginine (µmol/L)	0.6±0.2	02.0±0.6**

Note: * P < 0.05 as compared to group A subjects, ** P < 0.001 as compared to group B subjects

Table 3: Correlation of asymmetric dimethylarginine (ADMA) with different parameters in Group A participants

Biomedical Indicators	r- value	P –Value
Fasting Blood Sugar	0.204	0.090
Glycosylated hemoglobin	0.312	0.009*
Cholesterol level	0.523	0.0001*
Triglyceride level	0.439	0.0001*
High-density lipoprotein (HDL-C)	0.090	0.458
Low-density lipoprotein (LDL-C)	0.360	0.002*

* Correlation is significant at the 0.05 level (2-tailed)

Table 4: Correlation of asymmetric dimethylarginine (ADMA) with different parameters in Group B patients

Biomedical Indicators	r- value	P –Value
Fasting Blood Sugar	0.743	0.001*
Glycosylated hemoglobin	0.682	0.001*
Cholesterol level	0.204	0.090
Low-density lipoprotein (LDL-C)	0.508	0.001*
Triglyceride level	0.084	0.490
High-density lipoprotein (HDL-C)	0.288	0.016

* Correlation is significant at the 0.05 level (2-tailed).

Figure 1: Correlation between serum ADMA and Fasting Blood Sugar level in normal Control Group (r =0.204, p =0.090)

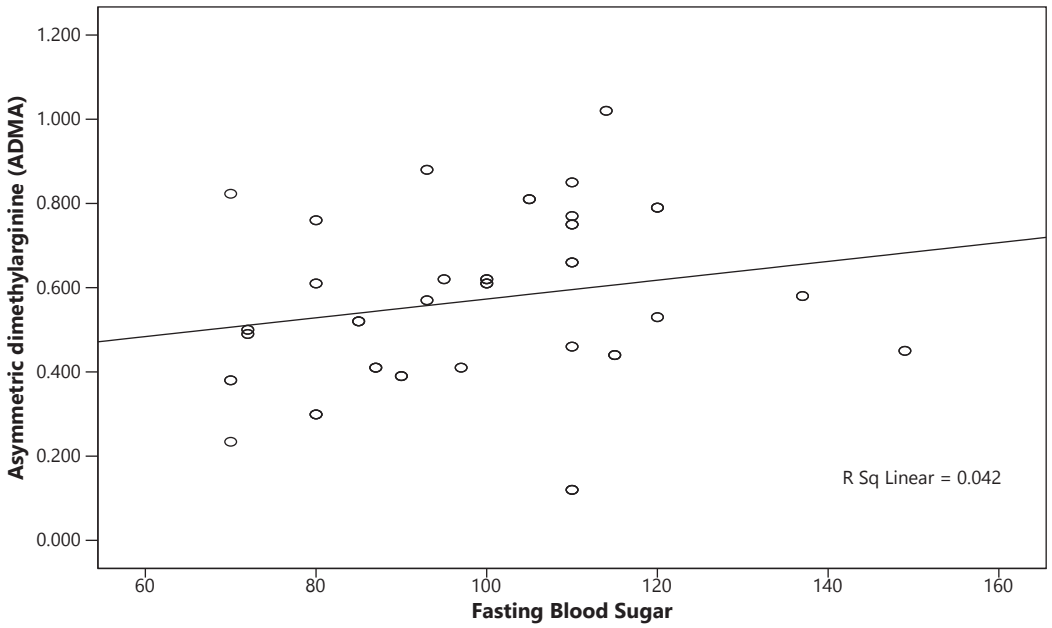


Figure 2: Correlation between serum ADMA and Fasting Blood Sugar level in type 2 diabetic patients (r = 0.743, p = 0.001)

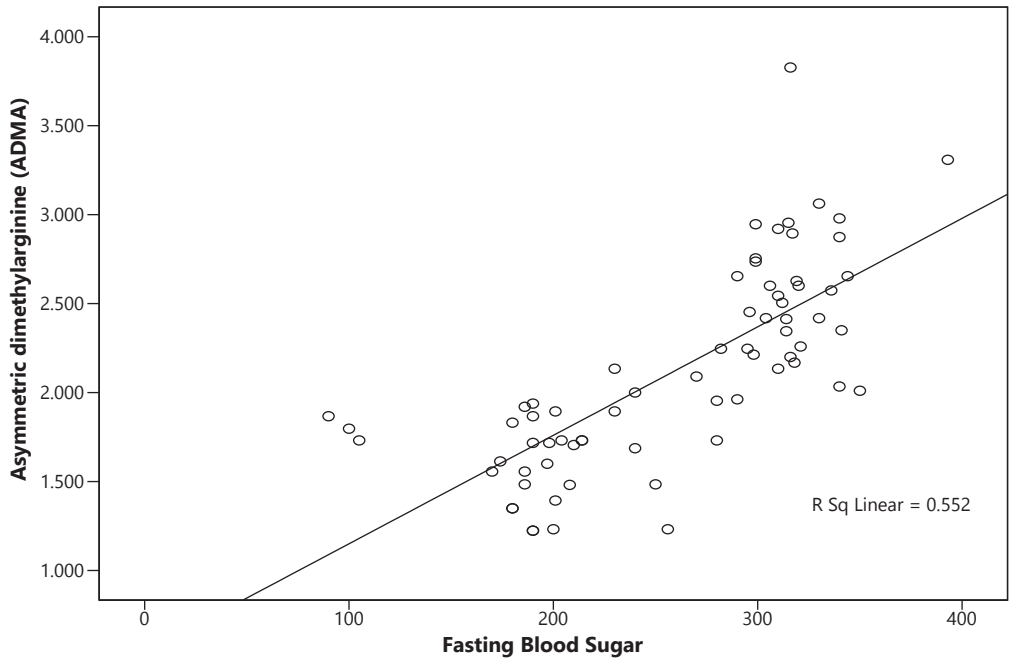


Figure 3: Correlation between serum ADMA and HbA1c (%) in type 2 diabetic patients (r = 0.682, p =0.0001)

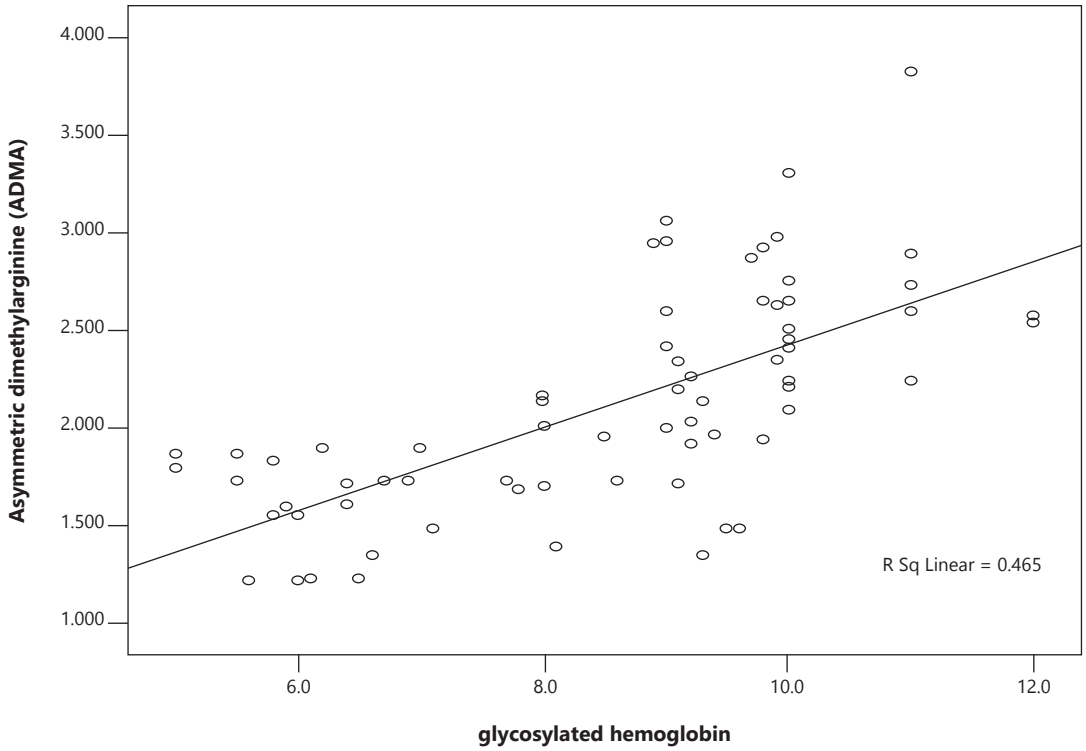
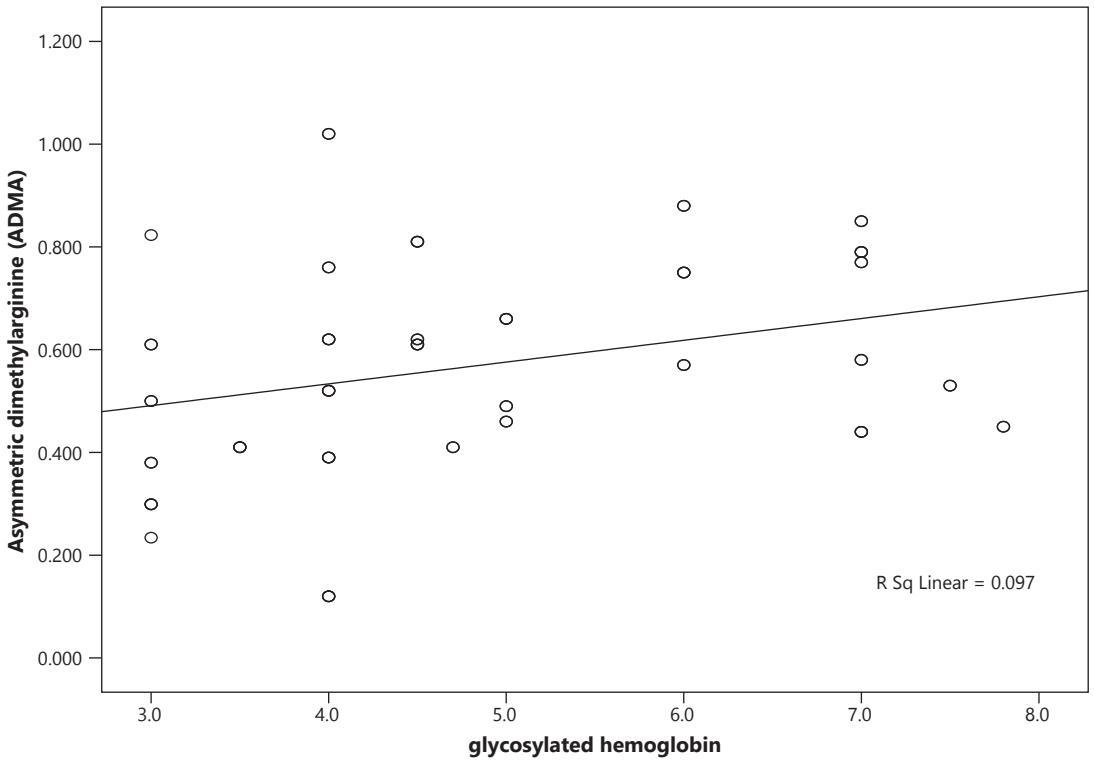


Figure 4: Correlation between serum ADMA and HbA1c in normal control group (r =0.523, p =0.001)



which is modified by oxidative stress, observed in patients with DM.

We found a significant positive correlation between serum ADMA level and HbA1c among group A and B. Devangelio and his colleagues[21] showed significantly higher ADMA levels in diabetic patients and found a direct correlation between HbA1c and ADMA levels. In contrast a study by Paiva et al[22] found ADMA inversely correlated with HbA1c. Xiong et al[23] observed no significant correlation between increased ADMA level and duration of illness of diabetes mellitus, but they found significant correlation between ADMA levels and glycemic control.

An increase in serum TC, TG and LDL-C levels were found among group B as compared to group A participants. These results are similar to study done by Gordon et al[24], Morton et al[25] explained that type 2 diabetic patients are usually associated with low plasma level of HDL and is often accompanied by elevated TG levels which poses an increased risk of developing endothelial dysfunction.

Sibel et al[26] showed the effect of statin therapy on serum ADMA level. They found that increased LDL-cholesterol levels were associated with increased serum ADMA level and this elevated level is associated with increased risk of endothelial dysfunction. Our study also showed increased serum level of ADMA and LDL in group B (167.6 + 38.7) as compared to group A (141.8 ± 36).

Another study conducted by Abbasi and his colleagues[27] showed that the levels of ADMA, plasma glucose and triglyceride were significantly higher in patients with type 2 diabetes mellitus.

Mahfouz et al[19] revealed ADMA as the potential risk factor in type 2 diabetes mellitus. They showed that the total cholesterol, triglyceride and LDL-C manifested significant elevations while HDL-C level showed insignificant change in type 2 diabetics as compared to non-diabetic healthy subjects. These findings were similar to the findings our study[19].

However, the exact mechanism of hyperglycemia influencing circulating ADMA concentrations in type 2 diabetes is not fully known. Increased blood glucose in type 2 diabetic patients induces oxidative stress, and so increases the plasma ADMA concentrations by impairing the dimethyl arginine dimethyl aminohydrolase (DDAH)[7,18,27].

CONCLUSION

In conclusion, our study reveals increased serum ADMA levels in patients with type 2 diabetes mellitus (group B) compared to control subjects (group A). Fur-

thermore significant positive correlation was seen between serum ADMA levels and FBS, HbA1c, TC, and TG level in type 2 diabetic patients. The results may suggest a possible role of long standing increased serum ADMA levels in development of complications of diabetes mellitus.

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CONTRIBUTORS

KT conceived the idea, did data collection and wrote the manuscript. MAK helped in data collection. Both authors contributed significantly to the final manuscript.