DEMOGRAPHIC DIFFERENCES IN ADMA IN TYPE 2DIABETES MELLITUS WITH AND WITHOUT CORONARY HEART DISEASE

Kalsoom Tariq[1], Shahnawaz[2], Naheed Khattak[3], Mudassir Ahmad Khan[3]

ABSTRACT

Objective: To compare the demographic differences in serum ADMA , lipid profile ,fasting glucose and HbA1c in type 2 diabetics with and without CHD with controls.

Methods: This cross sectional study included a total of 210 participants, of which 70 were normal individuals in group A,70 were having type 2 DM in group B and 70 were type 2 diabetics with CHD in group C. Blood obtained from the participants was used to determine serum ADMA, Lipid profile, fasting blood glucose and HbA1c.

Results: Increased levels of serum ADMA and other biomedical parameters were found in group B and C as compared to group A, however no significant difference was found between the three comparative groups with respect to age and gender.

Conclusion: There were no significant difference in demographic features between the two diseased groups as compared to the control group.

Keywords: Assymetrical dimethylarginine (ADMA), Coronary heart disease(CHD),Type 2 diabetes mellitus(T2DM), Glycosylated hemoglobin(HbA1c).

INTRODUCTION

ADMA is an endogenous NO synthase inhibitor and is produced as a result of arginine methylation of cellular proteins found inside the nucleus and released when hydrolysis of these proteins occur.[1] About 80% of ADMA is degraded by an enzyme dimetylarginine dimethlylaminohydrolase while 20% is eliminated through kidneys.[2] Increased levels of ADMA leads to decrease in NO synthesis by competing with L-arginine substrate for NO synthesis causing endothelial dysfunction.[3]

Type 2 diabetes mellitus is emerging as an epidemic and according to WHO the incidence of type 2DM will increase upto 300 million by 2025 affecting both genders equally [8]. The mortality and morbidity of DM is because of its complications arising as a consequence of metabolic disorder which causes hyperglycemia.[9,10] This hyperglycemia is responsible for increased tissue breakdown leading to increased serum ADMA level and also impairing the enzyme DDHA responsible for the degradation of 80% of ADMA.[6]

Coronary heart disease is the leading cause of death for men in the United States, killing 321,000 men in 2013—that's 1 in every 4 male deaths.[7]About 8.5% of all white men, 7.9% of black men, and 6.3% of Mexican American men have coronary heart disease found with raised levels of ADMA.[8] Between 70% and 89% of sudden cardiac events occur in men.[9] Although heart disease is sometimes thought of as a "man's disease," around the same number of women and men die each year of heart disease in the world. Despite increases in awareness over the past decade, only 54% of women recognize that heart disease is their number one killer.[9]

About 5.8% of all white women, 7.6% of black women, and 5.6% of Mexican American women have coronary heart disease again found to have raised levels of ADMA. Almost two-thirds (64%) of women who die suddenly of coronary heart disease have no previous symptoms.[7,9]

Patients with type 2DM have 2 to 4 times increased incidence of CHD[10]. ADMA is considered as an indicator of endothelial dysfunction because it is associated with hyperglycemia, insulin resistance, hypertension, atherosclerosis. There is a strong relationship between vascular disease in type 2 diabetics and ADMA.[11]

This study was undertaken to evaluate serum ADMA level and its association with different biochemical parameters considering demographic characteristics.

METHODOLOGY

This cross sectional study was conducted in the outpatient medical and cardiology departments of Khyber teaching hospital and hayatabad medical complex Peshawar from October 2010 to 2011. Ethical approval was taken from the ethical review committee of Khyber medical college Peshawar.

[1] Department of Biochemistry, KGMC, Peshawar
[2] Department of Biochemistry, NMC, Nowshera
[3] Department of Biochemistry, KMC, Peshawar

Address for correspondence:
Dr. Kalsoom Tariq
Department of Biochemistry, KGMC, Peshawar
Cell: 0348-2816944
Email: drkalsoomtariq@yahoo.com

Of 210 participants, 70 were included in each group, comprising group A-healthy individuals, group B-patients with T2DM for the last three years and group C-type 2 diabetic patients with CHD in the last seven days. The participants were aged between 35 to 65 years. The male to female ratio was 100/110. Informed consent was obtained from all the participants. Those having febrile illness, inflammatory diseases, renal, liver or thyroid diseases were excluded from the study similarly those on lipid lowering therapy or rennin angiotensin system (RAS) inhibitors were also excluded. BMI range of 25-29kg/m^2 for both genders was taken according to WHO[12]. After an overnight fasting, blood samples were collected using aseptic technique. The blood was centrifuged at 3000rpm and the serum was collected in eppindorf tubes for FBG, ADMA, TG, HDL and total cholesterol. A portion of blood sample was put into EDTA tubes for HbA1c estimation.

Serum ADMA level was determined using commercially available enzyme-linked immune sorbent assay(ELISA) kit by IBL international, Germany. FBG, total cholesterol, TG and HDL was measured using enzymatic colorimetric method while LDL was calculated using friedwald'sformula[13]. Glycosylated hemoglobin was analyzed using chromatographic colorimetric method.

For analysis of the data SPSS version 15 was used. Both descriptive and inferential statistics were used for result summarization. P -value of <0.05 was considered statistically significant. The correlation was found using Pearson's correlation coefficient.

RESULTS

Figure 1 shows that the age distribution of the participants was from 35 to 65years with the mean age of 53.73 ± 6.436 SD and median 55 years indicating that 50% of the patients were from above 55 years of age group, while a similar proportion was from below 55 years age group. 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%). The mean age was 50.8± 8.0 in group A, 54.4 ± 5.2 in group B and 56.0 ± 4.1 in group C.

Table 1 shows that the ratio of male and female amongst the study participants was 100 (47.6%) to 110 (52.4%) respectively. The three groups; diabetic patient without cardiac disease, diabetic patient with cardiac disease and the normal control group were having equal numbers. Among the 70 diabetic subjects, 26 (37.1%) were male and 44 (62.9%) were female while among patients who had diabetes mellitus with cardiac disease 37 (52.9%) were male and 33 (47.1%) were female and a similar male to female ratio was seen in the normal group as in diabetes mellitus with cardiac disease patients group. 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.

Figure 2 shows the area profile of the study participants, out of 210 study participants, 143 (68%) were from urban and 67 (32%) from rural areas. While selecting the comparative groups, effort was made to have a similar proportion of living status among the groups having the almost same annual income.

Table 2 shows the biochemical parameters of the study groups showing that as compared to the normal, the diseased groups (diabetic with and without coronary artery disease) had high blood pressure (systolic), body mass index (BMI), 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 and gender.

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.

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

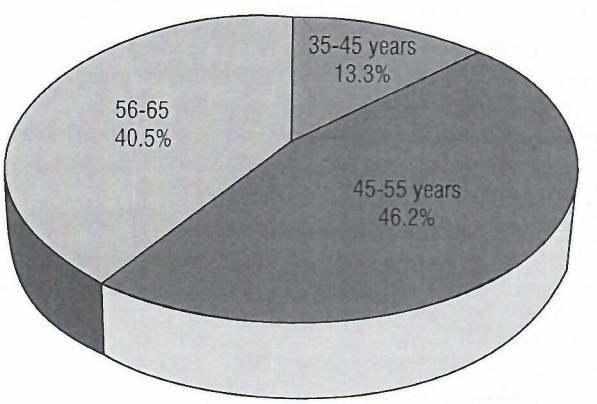

Figure 1: Age categorization of the participants

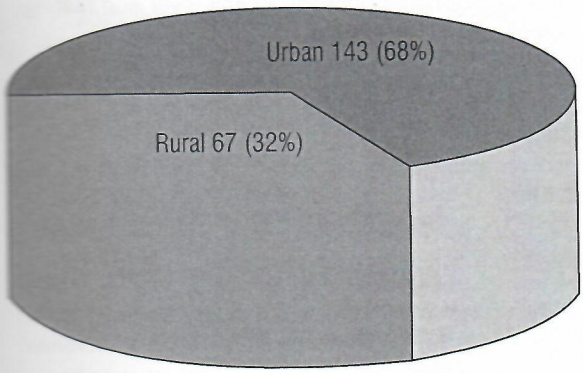

Figure 2: Area profile of the study participants

Table 1: Gender distribution of the participants

Study Groups	Gender	
	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%)

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.

DISCUSSION

Diabetes mellitus is a chronic metabolic condition in which the body stops producing or responding to insulin. As a result of this hyperglycemia occurs creating both short term and long term complications. Thus compared to non diabetics, people having diabetes are two to four times as likely to die from heart disease, two to six times as likely to have a stroke and four times as likely to have peripheral arterial disease resulting in atherosclerosis, diabetic retinopathy & neuropathy.[14] Sex-related differences in lifestyle may lead to differences in the risk of developing diabetes mellitus and its complications, to differences in the prevalence of this condition in women and men.[15]

WHO estimated that ischemic heart disease

Table 2: Biochemical parameters of the study participants

	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
Systolic blood pressure (mmHg)	119.0±10.8	154.4±21.6	151.6±25.7
Diastolic blood pressure (mmHg)	81.1±9.1	92.2±10.2	92.6±2.0
Body Mass Index (Kg/m^2)	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

contributed to a third of the 96.4% increase in the prevalence of heart failure from 1990 to 2013 worldwide.[16]

Accordingly, the European Society of Cardiology (ESC) identifies cardiovascular diseases as the leading cause of death in Europe. In a recent issue, the ESC estimated that, despite recent decreases in mortality rate in many countries, close to half deaths in Europe in 2014 are attributable to CVDs, with a higher proportion in women (51%) than in men (42%)[17].

The WHO also identified diabetes as the fifth and ninth cause of deaths in women and men, respectively. Indeed, diabetes is a major public health issue with an increasing prevalence globally, affecting at least 8% of the adult population worldwide[18].

Diabetes increases the prevalence of most of the main risk factors for CVDs, leading to an increased risk of related morbidity and mortality. However evidence have shown that women and men experience the disease differently because women lose their relative protection from CVDs, and postmenopausal diabetic females, compared to the general population, presented a stronger increase of cardiometabolic risk than diabetic males. The explanations are likely to be multifactorial, with contributions from differences in inherent physiological factors and in the management and treatment of diabetes, to the detriment of women[19].

Coronary artery disease is significantly associated with increasing age in T2DM patients according to the study of Larranaga et al.[20] In this study the mean age of majority of patients with T2DM having CAD was 52 years. Russo et al[21] also analyzed the association increase LDL with increase age as main risk factors for CAD in T2Diabetic patients. Similarly Sarwar et al [22] have also shown patients above 40s and 50s have two fold increase risk for CAD in T2DM. This corresponds well with this study, as majority of patients with T2DM with CAD belong to 45-55 years age range.

According to Rita[23] CAD in absence of diabetes was lower among female but in T2DM with CAD the ratio was found equal among both genders. Similarly the study of Nesto and his colleagues[24] showed both male and female are equally prone to develop CAD in Type 2 diabetic patients. This is in contradiction with this present study as majority of T2DM patients with CAD were males. This enlightens the fact that our male T2DM population is more prone to acquire all risk factors which will easily end in CAD.

This present study revealed, body mass index (BMI) among group B patients were more than normal Group (A) with significant statistical differences ($p < 0.0002$). This shows that T2DM patients with increase BMI have developed CAD. All these analysis corresponds well with the study of Romero and his colleagues.[25]

In this present study cardiac patients with diabetes presented with the highest mean systolic blood pressure than the normal group a P value 0f <0.0001. Similarly mean diastolic blood pressure was also raised in group B patients. It has been well documented in the study of Ravi and his colleagues [26]that prevalence of hypertension is higher among diabetic patients with CAD.

Cardiovascular disease (CVD) remains one of the major causes of premature death and disability in the UK and worldwide. Hypertension and diabetes are key risk factors for CVD, with their prevalence (in developed countries) being inversely related to socio-economic position.[27]

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 (2003).[28]

Duckelmann et al (2007) reported a positive correlation of serum ADMA concentration with fasting blood glucose level[29]. 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, ($r = 0.545$, $p = 0.001$). Devangelio et al (2007) 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.[30]

Yasuda et al (2006) conducted a study on type 2 diabetic patients with poor glycemic control, 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.[31] An increase in serum total cholesterol, triacylglycerol and low-density lipoprotein (LDL) levels were found among diabetic patients & diabetics with CAD as compared to normal healthy individuals.

CONCLUSION

This study reveals increased serum ADMA levels in both the diseased groups i.e type 2 diabetes mellitus without CAD and type 2 diabetes mellitus with CAD irrespective of the gender as compared to the normal healthy individuals. Also 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 Adjusting for age, sex, and social class, the prevalence of type 2 DM among urban subjects differ significantly from that among rural subjects. Increased age, higher socioeconomic class were proven to be independent risk factors for type 2DM &CAD in either area.

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