Evaluation of a Symmetric Dimethylarginine in Serum of Type 2 Diabetic Patients.

Zainab Abdul Wahed MSc

Shaymaa Zahraw Nada MSc

Isam Noori Selman CABM

Firyal H. Abdul-Jalil, MSc

Abstract

Background: Type 2 diabetes mellitus (DM2) is suddenly increasing in incidence, and is associated with increase risk for cardiovascular disease, because of the macrovascular and microvascular injury. Although the main physiological abnormalities are insulin resistance and impaired insulin secretion, the specific determinants of these metabolic defects remain uncertain. The experimental evidences suggest that inflammation may play an intermediary role in pathogenesis, there by linking diabetes with a number of commonly coexisting conditions thought to originate through inflammatory mechanisms. Serum asymmetric dimethylarginine (ADMA) level is biomarker of endothelial dysfunction and cardiovascular disease.

Aim: The aim of this study was to evaluate levels of ADMA, and its association with the complications of type 2 diabetic patients.

Materials and Methods: The ADMA serum levels were measured in 75 of type2 diabetic patients and 75 healthy control subjects by using high performance liquid chromatography (HPLC).

Results: A statistically significant differences was found in serum levels of ADMA between diabetic and healthy control groups ($p>0.001$). Serum levels of ADMA correlated significantly with the levels of (HbA_1c %) in type 2 diabetic patients.

Conclusion: These results suggest that serum ADMA levels directly contribute to the complications of cardiovascular disease in type 2 diabetic patients.

Key Words: type 2 diabetes mellitus, a symmetric dimethylarginine, (HbA_1c%) , lipid profile.

Introduction

Patients with type 2 diabetes mellitus have increased risk of atherosclerosis and its complications and frequently manifest postprandial hypertriglyceridemia, which has been shown to be an independent predictor of coronary artery disease.[1]

Endothelial cell dysfunction in large arteries is an important early event in the pathogenesis of atherosclerosis.[2] Abnormal endothelial cell–mediated vasodilatation has been demonstrated in patients with established atherosclerosis and in healthy subjects with risk factors for atherosclerosis as well as in patients with type 2 diabetes mellitus.[3] In patients with clinically evident atherosclerosis or risk factors for atherosclerosis, reduced endothelial cell–dependent vasodilatory responses of large arteries have been found and attributed to diminished synthesis of NO and/or to increased inactivation of NO in the vessel wall.[4] Endothelial cell–mediated brachial artery flow responses were reported to decline after ingestion of a high-fat meal in normal subjects and in patients with type 2 diabetes mellitus.[5]

In 1992, Vallance and colleagues[6] demonstrated that N^G,N^G-dimethyl-L-arginine(asymmetric dimethylarginine [ADMA]) is detectable in plasma and is an endogenous competitive inhibitor of NO synthase(NOS). N^G,$N^{G'}$-dimethyl-L-arginine (symmetric dimethylarginine [SDMA]) is also found in human plasma but does not inhibit NO synthesis.[6]ADMA is synthesized in many tissues, including vascular endothelial cells, and is thought to be derived from the hydrolysis of methylated proteins.[7] ADMA is excreted by the kidney, and elevated plasma levels have been found in patients with uremia. Elevated plasma levels of ADMA have been reported in animals and patients with hypercholesterolemia and with atherosclerosis [8] . The purpose of the present study was to investigate the changes in plasma ADMA levels that occur in subjects with type 2 diabetes mellitus .

Subjects &d Methods

Subjects:

1- Patients group:- seventy five patients (75)with type 2 diabetes mellitus with age range (30-60) years, Mean±SD (51.57±10.01) years were attended the National Diabetes Center, University of AL-Mustansiriya for treatment and research. The study conducted in March to September 2009.

All patients were on oral-hypoglycemic agents (i.e. metformin and/or glibenclamide). Patients with type 2 diabetes who received insulin injection were excluded from this study.

2-Controls group: Seventy five (75) apparently healthy subjects were involved as a controls group with matching age and sex to the patients group (mean age 47.6± 5.78) years.

None of the healthy subjects have a history of coronary heart diseases, renal failure, thyroid diseases or any metabolic known diseases that influence on carbohydrate metabolism.

Blood sample:
Twelve milliliters (12ml) of venous blood sample were taken, using plastic disposable syringes. Two milliliters were added to an ethylene diamine tetra acetic acid (EDTA) tube and ten milliliters were separated by centrifugation at (3000 rpm) for 15 min. the sera were stored frozen at (-20 ºC) until assayed
Each serum sample was analyzed for ADMA, total cholesterol(TC),triglyceride(TG),highdensitylipoprotein cholesterol(HDL-C), fasting blood sugar(FBS), and HemoglobinA1c (HbA_1c%).

Methods:
-Determination of serum asymmetric dimethylarginine (ADMA):
Serum asymmetric dimethylarginine (ADMA) levels were measured by reversed phase liquid chromatography (HPLC) after pre-column derivetization with ortho-phthalaldehyde (OPA) as described previously by Zeiger et al. 1992 [9].

-Determination of Haemoglubin A1c %(HbA1c %):
HbA_1c% was measured by variant TU (HbA_1c%) program, which intended for determination of (HbA_1c%) in human whole blood using Ion Exchange High Performance Liquid Chromatography (HPLC)[10].
The (75) diabetic patients distributed according to the level (HbA_1c%) into three groups depending on the American Diabetes Association (ADA) HbA_1c% guidelines :
Group 1: (HbA_1c%) level was less than 7% which is the goal of the treatment
Group 2: (HbA_1c%) level between 7%-8%, clinical intervention is suggested
Group 3: (HbA_1c%) level is more than 8%, clinical intervention is recommended.

-Enzymatic methods: were used for measuring Fasting blood sugar, Triglycerides, Total cholesterol, High density lipoprotein cholesterol and estimation of Low density lipoprotein cholesterol was done by the calculation method of Friedwald Formula[11], also very low density lipoprotein cholesterol was calculated by (TG/5).

Statistical analysis:
Statistical analysis was done using Excel system version 2003 and includes descriptive statistics (mean and standard deviation) and inferential statistics (t-test) to test the significancy of mean difference. When P-value was less than 0.05, the difference is considered statistically significant and the difference is considered highly significant when P-value was less than 0.001.

Results:
The results of serum levels of Fasting blood sugar and (HbA_1c%) for type 2 diabetes mellitus showed a significantly ($p<0.001$) higher difference when compared to the healthy controls. Results for serum lipid profile measurements for both DM & control groups are summarized in **table (1)**.

Table (1): Fasting blood sugar, (HbA_1c%) and lipid profile in diabetic and healthy control groups.

Biochemical testes	Control N=75	Diabetic N=75	t-test, P-value*
FBS(mg/dl)	81.75±11.92	227.94±65.01	0.001
HbA_1c%	3.80±0.82	10.38±1.92	0.001
HDL-C (mg/dl)	48.28±6.32	37.21±6.16	0.001
VLDL-C (mg/dl)	20.00±5.51	34.71±6.74	0.001
LDL-C (mg/dl)	84.84±20.31	284.76±135.16	0.001
Atherogenic index	1.780±4.186	8.171±4.186	0.001
Total Cholesterol (mg/dl)	153.12±23.10	356.69±136.94	0.001
Triglyceride (mg/dl)	100.00±27.54	173.56±33.68	0.001

There was a significant reduction of S.HDL-C in diabetic, while there was significant elevation in S.LDL-C, S.VLDL-C, total cholesterol, atherogenic index and triglycerides levels in diabetic when compared with healthy control groups (p=0.001). Asymmetric Dimethyl Argnine(ADMA) levels in the diabetic groups are show highly significant difference when compared with control group as shown in **table (2).**

Also there was a positive correlation between S.ADMA concentration and (HbA_1c%) for diabetic and healthy control groups as shown in **figure 1 and figure 2 respectively.**

Table (2): ADMA concentration (µmol/l)in all diabetic groups and healthy controls according to (HbA_1c%) levels .

Diabetic	n	ADMA µmol/l	t-test, P-value
HbA_1c < 7%	25	0.84±0.012	0.001
HbA_1c7%-8%	25	0.97±0.005	0.001
HbA_1c >8 %	25	1.31±0.031	0.001
Control	75	0.53±0.004	

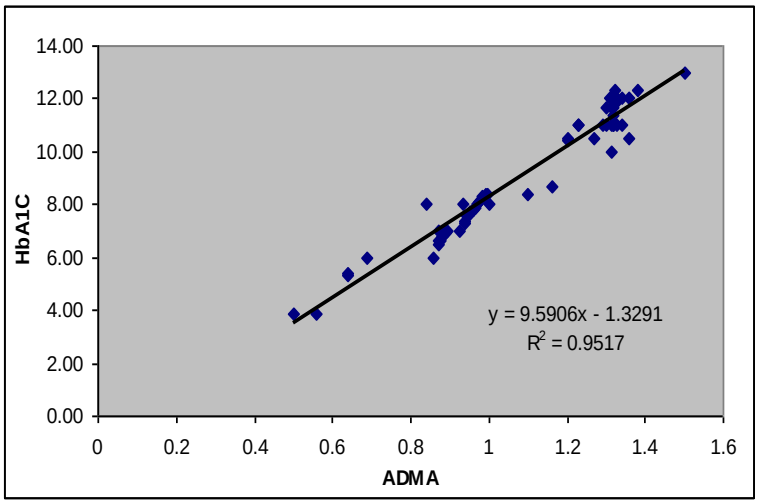

Figure (1): Correlation between ADMA level (µmol/l) and HbA_1c% in diabetic patients.

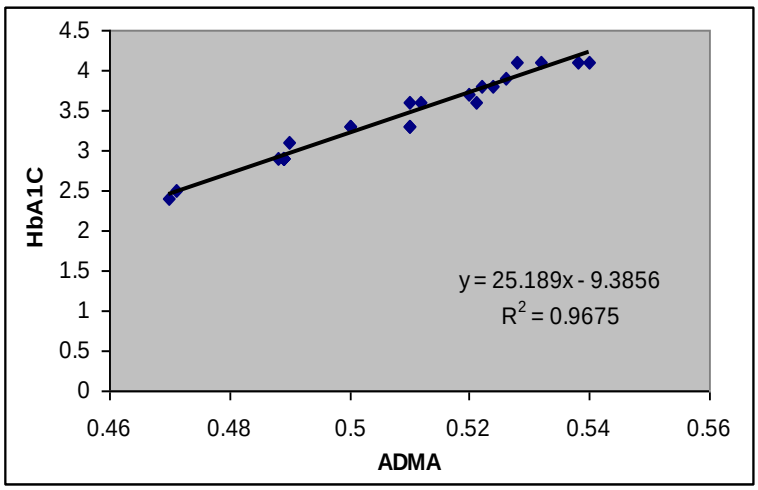

Figure (2): Correlation between ADMA level (µmol/l) and HbA_1c% in healthy controls.

Discussion

Diabetes mellitus is a heterogeneous, multifactorial and polygenic disease, characterized by insulin secretory defect of beta cell and insulin resistance in peripheral tissue which lead to elevated glucose levels[12].evaluation of the markers of endothelial function in type 2 diabetic patients is important in determining their susceptibility to cardiovascular disease. The results of this study demonstrated a significant elevation of ADMA in type 2 diabetic patients when compared with the well-matched controls. However, we found a significant correlation between serum ADMA levels and HbA_1c% in type 2 diabetic patients and healthy controls. Elevated ADMA level is accepted as an early marker of the initial stages of atherosclerosis [13]. It has been demonstrated that ADMA concentrations were elevated in patients with cardiovascular and metabolic diseases, including hypertension, lipid disorders, and diabetes mellitus [14] which are the components of metabolic syndrome.

Hyperlipidemia is one of the major risk factor for cardiovascular disease [15].According to the present results, there was a significant elevation of serum total cholesterol and serum triglycerides in patients with type 2 diabetes mellitus (p=0.001) when compared with healthy control subjects, and to reduce this increased risk, multifactorial approach to the management of type2 diabetes has been advocated. Recommended lifestyle interventions included reduce intake of dietary fat, regular participation in light or moderate exercise, and cessation.[16]

The oxidation of LDL is a very complex procedure [17]. The diabetic state alters LDL size and composition, LDL in postprandial state appears to be more susceptible to oxidation than fasting LDL.

Oxidation of LDL leads to alteration of the LDL apolipoprotein B (apo B) recognition site and in the unregulated uptake of the LDL by the macrophages via the scavenger receptor[18].

Another important factor in LDL oxidation relates to ambient HDL concentration. HDL carries important antioxidant enzymes, paroxanase and platelet activating factor acetythydrolase, and also it serves to protect LDL from oxidation in other ways. HDL also appears to exchange undamaged phospholipids for oxidized phospholipids in LDL. HDL from diabetic subjects is less protective against in vitro oxidation of LDL than HDL from non-diabetic control subjects[19]. Lipid profile, which is altered in the serum of diabetic patient, appears to be a significant factor in the development of premature atherosclerosis. The data of this study showed a significant elevation of LDL-C and a significantly reduction in HDL-C in patients with type 2 diabetes mellitus with p-value= 0.001 when compared with healthy control subjects, this in according with previous reports[20 21].

The present study suggests that serum ADMA levels directly contribute to the complications of cardiovascular diseases in type 2 diabetic patients.

References

1. Haffner SM, Lehto S, Ronnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med.* 1998; 339:229–219. .
2. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med.* 1999; 340:115–126.
3. Konishi H, Sydow K, Cooke JP.: Dimethylarginine dimethylaminohydrolase promotes endothelial repair after vascular injury *J*. Am. Coll. Cardiol .2007;49:1099-1105
4. Iribarren C, Husson G, Sydow K, Wang BY, Sidney S, Cooke JP. Asymmetric dimethyl-arginine and coronary artery calcification in young adults entering middle age: the CARDIA study Eur. *J*. cardiovasc. Prev. Rehabil.2007; 14:222-229.
5. Stuhlinger M, Abbasi F, Chu JW, Lamendola C, McLaughlin TL, Cooke JP, Reaven GM, Tsao PS.: Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor J. Am. Med. Assoc.2002;287:1420-1426.
6. Vallance P, Leone A, Calver A, Collier J, Moncada S. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. *J Cardiovasc Pharmacol*. 1998; 20(suppl 12):S60–S62.
7. Fickling SA, Leone AM, Nussey SS, Vallance P, Whitley GS. Synthesis of N^G,N^G-dimethylarginine by human endothelial cells. *Endothelium.* 2000; 1:137–140.
8. Böger RH, Bode-Böger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, Blaschke TF, Cooke JP. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. *Circulation.* 2007; 98:1842–1847.
9. Ziegler, F., Le-Boucher, J., Coundry-Lucas, C., Cynober, L.,: Plasma amino acid determination by reversed phase HPLC. Improvement OA method & comparison with ion- exchange chromatography. J-Autom-Chem, 1992; 14:145-149. Cited from Abdul-Rahman A.L. Al-Bazzaz. Serum amino acids & lipid profiles in well and poorly controlled patients with diabetes mellitus . Ph.D. thesis. 1997. Saddam College of medicine. Saddam University.
10. Rohfing CL: Use of HbA1c in screening for undiagnosed diabetes in the USA populations. Diabetes Care 2000; 23:187-191.
11. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low- density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem.1972;18:499-502.
12. Ferrannini E. Insulin resistance versus insulin deficiency in non insulin-dependent diabetes mellitus: problems and prospects. Endocr Rev 1998; 19:477-90.
13. Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, et al. Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis. Circulation1999; 99:1141-6.
14. Abbasi F, Asagmi T, Cooke JP, Lamendola C, McLaughlin T, Reaven GM, et al. Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. Am J Cardiol 2001; 88:1201-3.
15. Rafiei M, Boshtam, Sarraf-Zadegan N, Eastern Med Heal J.1999; 5:766.
16. Fiberg RM, Stella RH, Morimoto JM: Arq Bras Cardiol. 2001; 76:143.
17. Caren G: Reducing the Cardiovascular risk in T2DM. NEJM Editorial 2003; 348:457-459.
18. Jilal I, Devaraj S: The role of oxidized LDL in atherogenesis. J Nutr 1996; 126:1053S-1057S
19. Navab M, Berliner JA, Watson AD: The Yin and Yang of oxidation in the development of fatty streak. Atherioscler Thromb Vasc Biol 2005; 16:831-842.
20. Guerci B, Antebi H, Meyer L: Increased ability of LDL from normolipidemic type 2 diabetic women to generate peroxides. Clin Chem. 2006; 45(9):1439-1448.
21. Matsuguma, K.: Molecular Mechanism for Elevation of Asymmertic Dimethylarginine and Its Role for Hypertension in Chronic Kidney Disease. In: *J*. Am. Soc. Nephrol. 2006; 17(8), 2176-2183.

Al-Nahrain Medical College, Biochemist Dept.
Al-Mustansiriya Medical College, Diabetic Center.