Increased Levels of Asymmetric Dimethyl Arginine (ADMA) in Population at Risk For Cardiovascular Disease; A Study From Central India

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Summary

An endogenous inhibitor of nitric oxide synthase, asymmetric dimetylarginine (ADMA), is elevated in patients with type-2 diabetes mellitus (DM) and has been related to atherosclerotic disease. Little is known about the prognostic impact of serum ADMA determination in Indian population. This study was designed to evaluate the status of ADMA, nitric oxide (NO) and their possible correlation in patients with type-2 diabetes and cardiovascular disease in comparison to normal controls. The levels of ADMA, NO were measured in study population along with routine parameters using standard method and equipments. We found elevated ADMA (0.60±0.11µmol/L vs 0.53±0.10µmol/L, p <0.001) and decreased NO (32.14±6.4µmol/Lvs 47.37±8.88µmol/L, p <0.001) serum level in diseased group as compared to control; on intra group analysis in diseased group we found negative significant correlation between ADMA and NO (r= -0.747, p <0.001). Also no significant relation was found in between ADMA and cholesterol. In conclusion, the results of this study suggest that circulating ADMA adds independent prognostic information with regard to cardiovascular risk beyond that obtained from classical risk factors.

Key Words – Assymetric dimethylarginine (ADMA), Nitric oxide (NO), Diabetes mellitus (DM), Cardiovascular disease (CVD)

Running title: "ADMA independent predictor of CVD"

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LIST OF ABRÉVIATIONS USE

ADMA – Assymetric dimethyl arginine; **NO** – Nitric oxide ; **CVD** - Cardiovascular disease; **DM** – Diabetes mellitus; **NOS** – Nitric oxide synthase; **DDAH** – Dimethylarginine dimethylaminohydrolase; **TC** – Total cholesterol; **TG** – Triglyceride; **LDL-C** – Low density lipoprotein cholesterol; **HDL-C** – High density lipoprotein cholesterol; **VLDL-C** – Very low density lipoprotein cholesterol; **DwCVD** – Diabetes with cardio vascular disease; **BMI** – Body mass index; **BPS** – Blood pressure systolic; **BPD** – Blood pressure distolic

Introduction

Cardiovascular complications are the major cause of mortality and morbidity for the 135 million individuals worldwide afflicted by type 2 diabetes mellitus (DM).[1–3]
Endothelial dysfunction is a common feature in diabetic patients[4–6] and may contribute to cardiovascular morbidity.[7–9] Mechanisms of diabetes-induced endothelial dysfunction include the production of prostanoid vasoconstrictors and the increased oxidative degradation of NO.[10,11] Deficiency of NO increases vascular resistance and promotes atherogenesis.[12] In addition to its increased oxidative degradation, another possible mechanism for NO deficiency and cardiovascular morbidity is reduced NO synthesis caused by asymmetric dimethylarginine (ADMA).[13]ADMA is an endogenous competitive inhibitor of NO synthase (NOS).[14] This modified amino acid is derived from proteins that have been posttranslationally methylated and subsequently hydrolyzed.[15] ADMA is in part cleared by renal excretion.[16] Reduced clearance of ADMA in renal failure is associated with endothelial vasodilator dysfunction, reversible by administration of L-arginine[14,17] or by dialysis, which removes plasma ADMA.[18] However, the enzyme dimethylarginine dimethylaminohydrolase (DDAH) accounts for most of the clearance of ADMA.[19] DDAH metabolizes ADMA to L-citrulline and dimethylamine.[20] ADMA is elevated to a level that can significantly inhibit NOS activity in individuals with hypercholesterolemia, hypertension, hyperhomocyst(e)inemia, tobacco exposure, and hyperglycemia[21–23] of them hyperglycemias can elevate intracellular oxidative stress through multiple mechanisms.[24,25]
The serum concentration of ADMA, which could be used to monitor early changes in the L-arginine-NO-metabolism, has not been studied in Indian population having cardiovascular disease and type 2 diabetes. In the present study we investigate the relationship between serum ADMA levels and NO levels in a well selected group of type-2 diabetes with CVD and well matched healthy subject without diabetes and CVD.

Material and Methods

The study included total of 100 well matched healthy subject and 100 patients of the age 45-65 years suffering from type II diabetes and having positive findings of cardiovascular disease.
CVD patients: Angiographically proven patients by the cardiologists with relevant coronary artery disease showing >50% stenosis in at least one major coronary artery at the time of diagnostic catheterization was enrolled in this study. Each subject was screened by a complete history, physical examination and laboratory analysis.
Fasting blood samples were collected from the anticubiotal vein aseptically and the sample were used for analysis serum ADMA by using enzyme immunoassay for the quantitative determination of endogenous ADMA[26], Manufacture by DLD DIAGNOSTIK GMBH and serum nitric oxide (as nitrite) was estimated by method of Cortas NK[27]by using Quantichrome™ Nitric Oxide Assay kit based on colorimetric determination of NO at 540 nm. Statistical analysis of the work was carried by using student's-t test and chi square test. Results were expressed as mean SD. Probability values of $P<0.05$ were considered to indicate statistical significance.
Present work was approved by institutional ethical and research and development committee.

Results

Baseline data are shown in table-1. Diabetes subject with CVD had higher blood pressure (systolic), fasting sugar, total cholesterol, triglyceride, very low density lipoprotein and decreased high density lipoprotein cholesterol levels than normal healthy subjects.

Type 2 diabetes with CVD patients have significantly higher serum ADMA concentration than normal healthy subjects (0.60±0.11µmol/L vs 0.53±0.10µmol/L) p <0.001. Serum NO concentration were 32.14±6.4µmol/L in Diabetes with CVD patients and 47.37±8.88µmol/L in normal healthy subjects, there was a significant decrease in NO concentration in diseased group p <0.001.

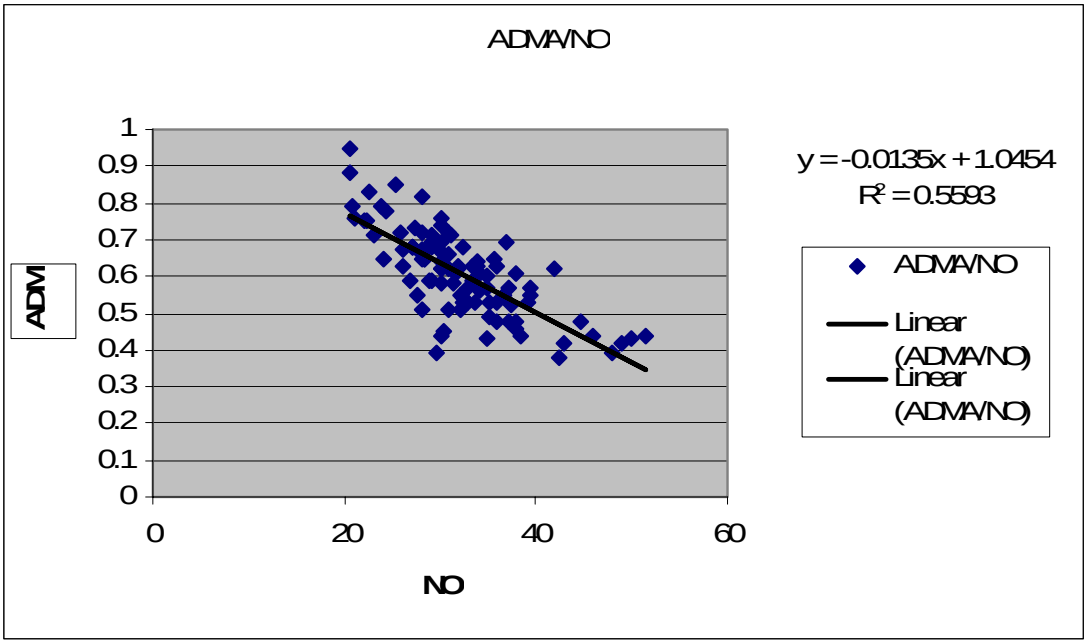
In figure-1 we report the relationship between ADMA and NO in diseased group, on intra group analysis there is significant negative correlation (r= -0.747, p <0.001) was found between ADMA and NO. It suggests that increase in ADMA associated with decreased NO activity or concentration.

Table-1: Baseline characteristics of the study population

	Control	Diseased	P value
Age (years)	45.29 ± 10.21	57 ± 7.50	< 0.10
Sex	6(F)	10(F)	-
Family History	16	21	-
Smoker	5	20	-
BMI	20 (>25)	30 (>25)	-
BPS (mmHg)	123.74 ± 3.24	130.82 ± 8.41	< 0.01
BPD (mmHg)	79.58 ± 6.53	84.99 ± 6.35	< 0.20
FS (mg/dl)	94.70 ± 19.13	129.80 ± 49	< 0.01
TC (mg/dl)	156.95 ± 27.68	271.41 ± 31	< 0.10
HDL (mg/dl)	42.24 ± 8.54	28.96 ± 9.69	< 0.01
LDL (mg/dl)	101.08 ± 27.95	158.68 ± 30.19	< 0.05
VLDL (mg/dl)	33.52 ± 11.39	44.31 ± 16.30	< 0.02
TG (mg/dl)	175.19 ± 66.91	226.16 ± 78.59	< 0.01
HbA1c (%)	6.5 ± 1.59	7.032 ± 2.30	< 0.50
ADMA (µmol/L)	0.530 ± 0.101	0.60 ± 0.11	< 0.01
NO (µmol/L)	47.37 ± 8.88	32.14 ± 6.40	< 0.01

BPS: Blood pressure systolic, BPD: Blood pressure diastolic, FS: Fasting blood glucose, TC; Total cholesterol, TG: Triglyceride, HDL: High density lipoprotein,
LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, HbA1c: Glycated haemoglobin, ADMA: Asymmetric dimethylarginine, NO:Nitric oxide

Figure-1: Correlation between ADMA and NO in diseased group.



Discussion

Type 2 diabetes is associated with increased risk of cardiovascular disease (CVD) in Asian Indians as compared to other subpopulation[28]. Hyperglycaemia is associated with endothelial dysfunction both in-vivo[29] and in-vitro [30] therefore endothelial dysfunction is an early feature in the development of vascular complications in people with diabetes [31].

The present study provide evidence that elevated serum ADMA and reduced No independently associated with cardiovascular risk in diabetes patients with coronary artery disease. Our study is consistent with previous studies[21,23,32] which shows elevated levels of ADMA in individuals with Type 1 and Type 2 diabetes, whilst contrasting with the results of another study reporting reduced levels in people with Type 2 diabetes compared to healthy individuals[33,34]. ADMA has been associated with many traditional and novel risk factors in the setting of atherosclerosis. In particular, hypertension, hyperlipidemia, and hyperhomocysteinemia[35,36,37]. In addition, they are elevated in conditions of peripheral artery disease, stroke and end-stage renal failure[38,39,40]. ADMA is generated from the hydrolysis of ubiquitous proteins containing methylated arginine residues. The nuclear protein arginine N-methyltransferase (protein methylase I) has been shown to methylate internal arginine residues in a variety of proteins. The methyl groups may be distributed symmetrically or asymmetrically to the guanidinium nitrogens of arginine, resulting in SDMA, N-monomethylarginine with ADMA being the predominant isomer[41]. These methylated arginines are excreted in the urine. In addition, the metabolism of ADMA and N-monomethylarginine, but not SDMA, occurs via hydrolytic degradation to L-citrulline and dimethylamine by DDAH. DDAH is an oxidant-sensitive enzyme[20] The decline in DDAH activity was strongly associated with elevated ADMA levels in the plasma in vivo and in the conditioned medium in vitro[42]. DDAH dysfunction hence seems plausible, especially in the setting of DM, in which hyperglycemia has been known to elevate oxidative stress[24,25].

Several pathways have been characterized to account for the increased production of free radicals in hyperglycaemia. For instance, elevated glucose may activate the polyol pathway, leading to the oxidation of sorbitol to fructose, coupled by the reduction of NAD^- to NADH[43,44]. The increased ratio of NADH/NAD^- may in turn promote free-radical production by activating xanthine oxidase and inactivating intracellular and extra cellular SOD. It is possible that these processes contribute to reduced DDAH activity or glucose-induced oxidative stress would impair DDAH activity. This observation is supported by study of Lin et al [45].

Endothelium-derived nitric oxide (NO) is the most potent endogenous vasodilator known, exerting its effect via stimulation of soluble guanylate cyclase to produce cyclic GMP [46,47,48]. NO is critical modulator of blood flow and blood pressure[49,50,51,52]. It is released by the endothelium in response to shear stress and plays an important role in flow-mediated vasodilation[49,50]. Endothelial release of NO opposes the vasoconstrictor effects of norepinephrine, endothelin, angiotensin II, and serotonin[12].

Pharmacological inhibition or a genetic deficiency of endothelial NO synthase (NOS) impairs endothelium-dependent vasodilatation and increases vascular resistance[51,52,12,53] in patients with coronary artery disease, an impairment of NO activity may contribute to ischemic syndromes[54,55].Vascular NO activity is reduced in diabetes, leading to impaired endothelium-dependent vasodilation[56] and elevated platelet aggregation[57,58]. Asymmetric dimethylarginine (ADMA) is an endogenous and competitive inhibitor of nitric oxide synthase. Plasma levels of this inhibitor are elevated in patients with atherosclerosis and in those with risk of atherosclerosis. In these patients, plasma ADMA levels are correlated with the severity of endothelial dysfunction and atherosclerosis. By inhibiting the production of nitric oxide, ADMA may impair blood flow, accelerate atherogenesis, and interfere with angiogenesis[59].

Thus, reduced NO levels seem to play a central role in the development of endothelial dysfunction amongst the multiple pathogenetic mechanisms that have been postulated. NO levels might be reduced as a result of a combination of decreased NO production because of decreased activity or reduced expression of eNOS, or its increased degradation by reactive oxygen species or increased production of super oxide ions.

Accordingly, treatment of these cells with antioxidants has been demonstrated to restore the activity of dimethylarginine dimethylaminohydrolase[60,22], leading to a normalization of cellular ADMA levels and endothelial nitric oxide production. Similar in vivo study of L-arginine and antioxidant supplementations has shown increase in NO production and improved endothelial function in Indian population[61].

Thus, increased synthesis of ADMA and the subsequent impairment of nitric oxide synthesis may provide a common pathway by which many of the proatherogenic factors leads to clinically relevant cardiovascular risk. Since concentration of ADMA acts as a marker (or even producer) of endothelial dysfunction[62], circulating levels of ADMA have been related to presence, extent, and severity of coronary artery disease[63].

The data of our study demonstrate for the first time in our population that ADMA is associated with CVD risk and the strength of ADMA for CVD risk prediction was shown in comparison with traditional marker. Which are used extensively in routine clinical practice. In the present cohort, ADMA proved to provide independent predictive power than other routine markers. In recent studies, a strong correlation of ADMA with serum cholesterol has been described[64], but the present investigation cannot confirm the same and we found weak insignificant correlation.

In present study circulating levels of ADMA have been analyzed by using recently introduced ELISA technique that has been extensively evaluated against liquid chromatography mass spectrometry[65]. It represents a reliable procedure that is suitable for the determination of ADMA in large sample series using easily available equipment ELISA reader in developing country like India. From a clinical perspective, assessment of ADMA might aid cardiovascular risk assessment. This biomarker represents nitric oxide bioavailability oxidative stress also and thus can identify individuals at high cardiovascular risk even in an early stage, apart from traditional risk factors. In conclusion, the results of this study suggest that circulating ADMA adds independent prognostic information with regard to cardiovascular risk beyond that obtained from classical risk factors.

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