Correlation of Asymmetric Dimethylarginine with Cardiovascular Disease in Prediabetes

Santosh Bidwe[1], Prashant Hisalkar[2], Neerja Mallick[3]

Abstract

Asymmetric dimethylarginine (ADMA) is now well established as a major risk factors for cardiovascular disease (CVD) impact upon endothelial function by decreasing nitric oxide (NO°) bioavailability. Asymmetric dimethylarginine, an endogenous analog of L-arginine, is able to inhibit the activity of endothelial-nitric oxide synthase (eNOS), promoting endothelial dysfunction. Prediabetes is characterized by a reduced endothelium-dependent vasodilation and increased ADMA levels. Asymmetric dimethylarginine is strongly associated with micro- and macrovascular diabetic complications. Asymmetric dimethylarginine activity is strongly correlated with CVD in prediabetes.

Materials and methods: This study was a cross-sectional, descriptive type of study. In total, 815 participants were involved in this study, out of which 250 suffered from type II diabetes and 265 were prediabetic patients. 290 controls were involved from hospital OPD. Biochemical parameters including fasting plasma sugar, postprandial plasma sugar (after 2 hours of 75 g oral glucose), fasting lipid profile (serum total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, triglycerides (TG), high-density lipoprotein (HDL) cholesterol, very low-density lipoprotein (VLDL) cholesterol) were done by enzymatic methods. The quantitative sandwich enzyme immunoassay technique was used to determine plasma ADMA level by using commercially available enzyme-linked immunosorbent assay.

Results: The level of ADMA in prediabetes was 0.55 ± 0.11 and of type II diabetes was 0.70 ± 0.14 compared with controls (0.41 ± 0.14). p value was <0.05, which was significant. In the present study, there was a significant increase in serum TC, TG, LDL, VLDL, TG/HDL, and LDL/HDL ratio compared with those of normal healthy subjects, while HDL was significantly decreased in prediabetic as compared to normal healthy subjects.

Conclusion: The current study shows that increased ADMA levels can indicate the risk of CVD in prediabetic stage. Prediabetes people are under risk of CVD and type II diabetes. Individuals who are prediabetic are at risk of CVD and type II diabetes. The evaluation of the ADMA levels may improve the early diagnosis of CVD of prediabetes.

Keywords: Asymmetric dimethylarginine, Cardiovascular disease, Nitric oxide, Prediabetes, Type II diabetes.

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Introduction

Diabetes, which is defined as a fasting blood glucose level (FBGL) of greater than 6.9 mmol/L[1] is associated with extensive organ dysfunction including diabetic retinopathy, kidney disease, and CVD, gastrointestinal disturbance, sexual dysfunction, and diabetic neuropathy.[2,3] The fatal macrovascular complications account for the majority of deaths among patients with diabetes.[4] Risk of CVD is already increasing in prediabetes patients, i.e., impaired glucose tolerance (IGT) patients who are defined as having FBGL of 5.6–6.9 mmol/L. Some studies represent that IFG is not just a precursor of diabetes, but an individual risk factor for death. The incidence of prediabetes is increasing globally to probably more than 400 million cases in 2030 and, if untreated, will progress to diabetes and the associated complications.[5]

Endothelial dysfunction was indicated as a major cardiovascular risk factor in various trials.[3] Endothelial dysfunction is defined as a decrease in bioavailability of NO°, which inhibits the adhesion and aggregation of platelets, vascular smooth muscle cell proliferation, and LDL oxidation, and adhesion of monocytes and leukocytes to the endothelium.[4] Asymmetric dimethylarginine is essentially a competitive inhibitor of eNOS. Asymmetric dimethylarginine regulates the production rate of NO°. Asymmetric dimethylarginine is degraded by enzyme dimethylarginine dimethyaminohydrolase (DDAH) plasma ADMA concentration has been shown to increase during the course of diseases associated with endothelial dysfunction such as diabetes mellitus, peripheral artery disease, hypertension, and CVD.[6–10]

[1]Department of Biochemistry, SMBT Institute of Medical Sciences and Research Centre, Nashik, Maharashtra, India

[2]Department of Biochemistry, Government Medical College and Hospital, Dungarpur, Rajasthan, India

[3]Department of Biochemistry, People's University, Bhopal, Madhya Pradesh, India

Corresponding Author: Prashant Hisalkar, Department of Biochemistry, Government Medical College and Hospital, Dungarpur, Rajasthan, India, Phone: +91 9422610220, e-mail: pjhisalkar@yahoo.co.in

Cardiovascular disease is a large account fraction of morbidity and mortality in type II diabetes.[11] Most of the studies were done on ADMA. Risk factors for CVD were clinically clear in diabetes mellitus, but it is not clear whether increased concentrations of ADMA are present in patients with IGT. In our view, there are hardly any studies available in Indian population regarding these biomarkers in prediabetes. The aim of present study was to evaluate serum ADMA level in prediabetes and type II diabetes patients and find the correlation with other variables.

MATERIALS AND METHODS

This study was done in the Department of Biochemistry, People's College of Medical Science and Research Center (PCMS and RC), Centre for Scientific Research and Development (CSRD), People's University, Bhopal. This is a cross-sectional descriptive study includes 250 type II diabetes patients, 265 prediabetes persons, and 290 controls during the period June 2017 to April 2019. Written informed consent was taken from all participants after applying inclusion and exclusion criteria. Sociodemographic data were collected by a self-designed questionnaire.

Inclusion Criteria for Prediabetes According to American Diabetes Association

- Age: between 18 years and 60 years
- Fasting blood sugar level: 100 mg to 125 mg
- HbA1c: 5.7% to 6.4%
- Postprandial blood sugar level (after 2 hours of 75 g oral glucose): 140 to 199 mg/dL

Exclusion Criteria for Prediabetes

- Age more than 60 years and age less than 18
- Diagnosed diabetic patients
- Pregnant women
- HIV-positive patients

Inclusion Criteria for Type II Diabetes According to American Diabetes Association

- Age: between 18 years and 60 years
- Known case of type II diabetes (1–5 years) and HbA1c 6.5%

Exclusion Criteria for Type II Diabetes

- Age not more than 60 years and age less than 18
- Pregnant women, HIV-positive patients
- Prolonged diabetes (>5 years)
- Patients on statin therapy

The study protocol was approved by Institutional Ethics Committee. All the participants were screened for age, gender, fasting glucose level, postprandial glucose level, HbA1c, family history, and any medication history. Prediabetic cases were included and excluded with the help of physician, Department of Medicine, PCMS and RC. Biochemical parameters investigations are as follows (Table 1):

Statistical Analysis

The analysis was done using statistical package SPSS 24 and Microsoft Excel 2010. Independent t test was used for comparison. "p" value <0.05 was used for level of significance.

RESULTS

A total of 815 subjects participated, and out of these 255 were type II diabetes patients, 270 were prediabetes, and 290 were controls in the present study.

The level of ADMA in prediabetes was 0.55 ± 0.11 and type II diabetes was 0.70 ± 0.14 compared with controls 0.41 ± 0.14. p value was <0.05, which was significant (Table 2 and Fig. 1). Mean score of cholesterol in prediabetes was 243.34 ± 20.03 and type II diabetes was 311.92 ± 60.86 compared with controls (170.81 ± 22.34). p value was <0.001, which was significant. Mean score of triglyceride in prediabetes was 169.89 ± 14.33 and type II diabetes was 191.32 ± 40.96 compared with controls (91.11 ± 16.59). p value was <0.001, which was significant. Mean score of HDL in prediabetes was 31.10 ± 4.33 and type II diabetes was 27.97 ± 4.96 compared with controls (42.19 ± 5.82). p value was <0.001, which was significant. Mean score of LDL in prediabetes was 172.09 ± 20.03 and type II diabetes was 236.96 ± 56.27 compared with controls (109.06 ± 18.61). p value was <0.001, which was significant. Mean score of VLDL in prediabetes was 36.07 ± 7.04 and type II diabetes was 47.56 ± 10.45 compared with controls (19.04 ± 4.83). p value was <0.001, which was significant. Mean score of TG/HDL in prediabetes was 5.58 ± 1.00 and type II diabetes was 7.23 ± 2.22 compared with controls (2.19 ± 0.49). p value was <0.05, which was significant. Mean score of LDL/HDL in prediabetes was 5.74 ± 1.08 and type II diabetes was 8.94 ± 3.43 compared with controls (2.61 ± 0.56). p value was <0.001, which was significant (Table 2).

DISCUSSION

It is well recognized that type II diabetes and its metabolic derangements such as hyperinsulinemia, hyperglycemia, dyslipidemia, and increased oxidative stress are associated with NO-mediated endothelial dysfunction.[19] In contrast, endothelial dysfunction may lead to attenuated glucose uptake in insulin-sensitive tissues, hyperglycemia, and ultimately to the development of insulin resistance and type II diabetes.[20,21] Furthermore, impaired NO° bioavailability plays a pivotal role in the regulation of glucose-stressed endothelial progenitor cell dysfunction in type II diabetes, and antioxidant treatment with superoxide dismutase may restore their function.[22] ADMA, an endogenous competitive inhibitor of NO° synthase, is known to impair NO° bioavailability and endothelial function.[23] Overexpression of DDAH, the enzyme

Table 1: Biochemical parameters of inclusion criteria for Prediabetes

S. no.	Biochemical parameters	Method
1	Blood glucose	GOD–POD method[12]
2	Cholesterol	CHOD–POD method[13]
3	TG	Glycerol phosphate oxidase–peroxidase (GPO–POD method)[14]
4	HDL	Directly enzymatic colorimetric quantitative determination[15]
5	LDL	Friedewald equation assuming that TC is composed primarily
6	VLDL	By calculation
7	HbA1c	Enzymatic method[16]
8	ADMA	ELISA method[17,18]

Table 2: Comparison of endothelial dysfunction biomarkers in prediabetes and type II diabetes compared with controls

Variables	Control	Prediabetes	Type II diabetes	p value
Age	37.46 ± 10.42	45.70 ± 8.98	43.09 ± 10.88	<0.001
Gender (M/F)	159/131	168/102	148/107	–
HbA1c (%)	5.07 ± 0.35	6.01 ± 0.19	8.2 ± 1.19	<0.001
Cholesterol mg/dL	170.81 ± 22.34	243.34 ± 20.03	311.92 ± 60.86	<0.001
TG mg/dL	91.11 ± 16.59	169.89 ± 14.33	191.32 ± 40.96	<0.001
HDL mg/dL	42.19 ± 5.82	31.10 ± 4.33	27.97 ± 4.96	<0.001
LDL mg/dL	109.06 ± 18.61	172.09 ± 20.03	236.96 ± 56.27	<0.001
VLDL mg/dL	19.04 ± 4.83	36.07 ± 7.04	47.56 ± 10.45	<0.001
TG/HDL	2.19 ± 0.49	5.58 ± 1.00	7.23 ± 2.22	<0.05
LDL/HDL	2.61 ± 0.56	5.74 ± 1.08	8.94 ± 3.43	<0.001
ADMA (µmol/L)	0.41 ± 0.14	0.55 ± 0.11	0.70 ± 0.14	<0.05

$p < 0.05$ is consider as statistically significant
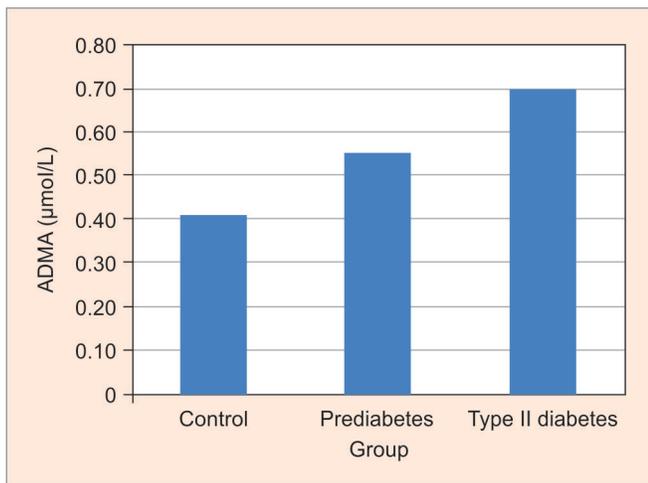
Fig. 1: Mean score of ADMA

responsible for the elimination of ADMA in human, may lead to reduced plasma ADMA level and enhance insulin sensitivity.[24] In human studies, insulin resistance is related to plasma ADMA concentration, impaired ability of enzyme DDAH, and treatment with rosiglitazone, and weight reduction enhances insulin sensitivity and significantly lowers plasma ADMA level.[25,26] Plasma ADMA level has been reported to be correlated with treadmill stress test-derived outcome parameters[27] and asymptomatic carotid atherosclerosis.[28] In prediabetic subjects, elevated ADMA was strongly associated with increased arterial stiffness, which is a well-established predictor of cardiovascular outcomes.[29] Hyperglycemia is associated with endothelial dysfunction both *in vivo*[30] and *in vitro*;[31] therefore, endothelial dysfunction is an early feature in the development of vascular complications in people with diabetes. These findings suggest that the elevated ADMA in diabetes could contribute to accelerated atherosclerosis in this population.[32]

Studies comparing ADMA levels of prediabetic subjects with healthy people are inconclusive. Although some studies report increased ADMA levels in prediabetes.[33] Eliana et al. report no significant difference between groups. Associations between increased levels of ADMA and many cardiovascular risk factors such as age, hypertension, diabetes, insulin resistance, hypercholesterolemia, hypertriglyceridemia, smoking, and BMI

have been accepted.[34] In our study, we found that ADMA levels were elevated in prediabetes and type II diabetes compared with healthy controls.

In our study, plasma ADMA levels were significantly increased in the prediabetes and type II diabetes compared with the control group. We suggest that increased ADMA levels could contribute to impaired endothelial function in prediabetic stage. Recent studies have indicated that LDL particle concentrations, and specifically LDL levels, are predictors of coronary events, and it is an independent coronary disease risk factor.[35] At the molecular level, high LDL-C levels induce endothelial cell dysfunction with subsequent decreased NO° bioavailability due to impaired L-arginine transport and metabolism and eNOS uncoupling. Peroxynitrite, the major uncoupling byproduct of eNOS, has been reported to directly damage elastin.[36] Studies show that subjects with the metabolic syndrome and/or DM have higher sLDL levels than controls.[37,38] In our study, we find a significant difference in serum lipid profile levels between the groups.

Conclusion

The current study shows increased ADMA levels can indicate the risk of CVD in prediabetic stage and type II diabetes. The evaluation of the ADMA levels may improve the early diagnosis of CVD in prediabetes patients. Along with routine investigations, an early detection of ADMA will help the clinicians to plan line of treatment in a better manner. Well-balanced nutrition, patient education, diet counseling, and supplementation therapies for high risk group of diabetic patients are strongly recommended.

The outcome of our study is to know the role of ADMA in prediabetes and diabetes in clinical manifestations of the disease, which ultimately helps us in better management of the patient. This study will help us develop the criteria for preventive measures of the disease using biochemical markers in predicting the disease.

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