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Brachial Artery Flow Mediated Vasodilatation Changes in Endothelium Due to Hyperuricemia in Type 2 Diabetes Mellitus

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Authors' contributions

Author PS performed the biochemical assays and assisted in writing the paper. Author PC analyzed the results and wrote the paper. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: This study aims to evaluate serum uric acid levels and assess its effect on endothelial dysfunction by measuring flow mediated vasodilatation (FMD) of brachial artery in type 2 diabetes mellitus.
Study Design: Observational and prospective
Place and Duration of Study: The study was carried out in Department of Biochemistry and Department of Radiology, MGM Medical College, Navi-Mumbai from August 2010 to 2012
Methodology: Total 90 patients were selected and divided in to three groups. Group I (n=30) – Controls, Group II A & B (n=60) –Diabetic patients without & with hyperuricemia (HUA). All subjects were examined by high resolution ultrasound to measure FMD. Serum uric acid and nitric oxide (NO) levels along with other biochemical parameters were estimated.
Result: Group II B showed significantly increased serum uric acid levels ($p<0.001$) along with decreased levels of serum NO ($p< 0.0001$) and decreased vasodilatation when measured by FMD. ($p<0.001$).
Conclusion: The FMD of brachial artery along with serum NO levels are reduced in patients of type 2 diabetes mellitus with HUA. Uric acid may be a contributing factor to endothelial dysfunction in type 2 diabetes. Such endothelial damage may be preventable by regularly monitoring uric acid levels and pharmacologically treating HUA.

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Keywords: Hyperuricemia; nitric oxide; endothelial dysfunction; type 2 diabetes mellitus.

1. INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disorder in man, currently affecting over 170 million people world-wide and is expected to affect 365 million by the year 2030 [1]. Type 2 DM is rapidly emerging as one of the greatest global health challenges of 21st century due to complications like ischemic heart disease, neuropathy, retinopathy and nephropathy. People with type 2 diabetes almost invariably have abnormal endothelial function as determined by the assessment of vascular reactivity and/or by the measurement of plasma markers of endothelial activation, coagulation/ fibrinolysis, or inflammation. They have been consistently found to have abnormal small and large vessel reactivity for both endothelium-dependent and independent vasodilatory pathways, demonstrating that there is not only a reduction in nitric oxide production in diabetes but also a decreased response to its effect in vascular smooth muscle cells [2]. There are many factors that contribute to the health of the endothelium. One factor is the uric acid level, which is commonly increased in diabetic patients[3]. Decreased NO level, another contributing factor, may either be due to decreased eNOS activity or decreased bioavailability. Hyperuricemia (HUA) decreases NO production in the endothelium through the inhibition of arginine transport. This in turn affects the insulin-mediated glucose uptake in the endothelium. Therefore HUA, along with decreased NO level can lead to increased insulin resistance (IR) along with endothelial damage.

The importance of HUA and clustering phenomena of metabolic syndrome (such as hypertension, hyperglycemia and HUA) were first described by Kyllin in1923.[4] Nagakwa in 2006 showed that uric acid plays an important role in the pathogenesis of metabolic syndrome probably due to its ability to inhibit endothelial function through decreasing NO bioavailability. He also hypothesized that HUA may have a key role in insulin resistance.[5] This study aims to assess the interplay of HUA, decreased NO production, and endothelial damage in diabetes. The extent of endothelial damage was evaluated non-invasively by flow mediated brachial artery vasodilatation (FMD).

2. MATERIALS AND METHODS

This is a prospective study conducted at MGM Medical College & Hospital at Kamothe, Navi-Mumbai. A total of sixty patients with type 2 Diabetes mellitus, between 35 and 70 years of age, from the outpatient Diabetic Clinic of MGM Medical College & Hospital were included in this study. Thirty age and sex-matched healthy individuals without any history or symptoms of diabetes were also included as controls. Patients were maintained on their usual pharmacological regimen for glycemic and blood pressure control as prescribed by their attending clinicians. A written, informed consent was taken from each participant.

The study was conducted after obtaining approval from the Institutional Ethics Research Review Committee. The subjects were divided mainly in two groups; Group I - Controls (n=30) & Group II - Diabetic patients (n=60) which were further divided in to two subgroups A & B based on the serum uric acid values (normouricemia and hyperuricemic, respectively). Pregnant patients and those with major illnesses were excluded from the study. The diagnosis of diabetes mellitus was based on World Health Organization criteria, i.e. a fasting blood glucose more than 126 mg/dl (7.0 mmoles/L) as confirmed by attending diabetologist.

For biochemical investigations, a fasting un-hemolyzed blood sample (after a minimum of 12 hours of fasting) was collected and used. All estimates (except that for NO) were done using commercial kits on an auto analyzer. LDL was calculated using the Friedewald formula. [6] Serum NO was determined indirectly by measuring the stable decomposition product (NO_2^-), employing Griess reaction according to the modified method of Mirinda et al.[7]

2.1 FMD (Flow Mediated Vasodilatation) Measurement of Brachial Artery

All study subjects were referred to the vascular laboratory in the morning for FMD measurement of brachial artery. FMD was studied by high-resolution peripheral vascular ultrasonic imaging as described previously [8,9]. Two-dimensional (2D) images of the left brachial artery were obtained using a high frequency 5-7 MHz linear transducer.Imaging was performed in a dimly lit, quiet room with the temperature maintained at 22–25ºC. Patients were made to rest in supine position for at least 10 minutes before the first scan and remained in the same position until the final recording. Blood pressure measurement was taken from right arm before imaging. Images were obtained 3–5cm above antecubital fossa of left arm. Baseline 2D images were acquired. To induce hyperaemia, a 5-6 inch wide blood pressure cuff was inflated at the forearm to 200 mmHg. Arterial occlusion was maintained for 2 minutes, with the transducer positioned carefully in an identical position. The cuff was then rapidly deflated, and 2D images of the brachial artery were recorded at 15 second intervals.

3. STATISTICAL ANALYSIS

ANOVA and post hoc test were applied for multiple comparisons of various parameters among the study groups. Linear regression analysis (Person correlation coefficient, r) was performed for determining the degree of association between different parameters. All values are expressed as mean ± SD, and p values of <0.001 were considered to be statistically significant.

4. RESULTS

Descriptive statistics of subjects in the control and the patient groups as well as comparative analyses of different parameters along with p value are presented in Tables 1 & 2. Male and female subjects were matched for age and number in both the control and the study groups. Table 2 compares the two diabetic subgroups (i.e. normouricemia and hyperuricemia).

All values in Table 1 and 2 are expressed as means and standard deviation. Blood glucose and lipid profile values were deranged in both the study groups (IIA and IIB). Serum NO and FMD was significantly lowered in Group II B in comparison with controls and Group IIA. This highlights the hypothesis that HUA is associated with NO non availability.

Table 1. Descriptive and comparative statistics for different groups by Tukey's POST-HOC test

Clinical Parameter	Control (Group I) (n =30)	Diabetic Normouricemic (Group II A) (n =30)	P value Control vs Diabetic Normouricemia	Diabetic Hyperuricemic (Group II B) (n =30)	P value Control vs Diabetic Hyperuricemia
	Mean ± SD	**Mean ± SD**		**Mean ± SD**	
Age in yrs	51.96 ± 4.18	50.80±4.97	0.664	51.80± 6.30	0.992
Weight (kg)	70.73 ± 2.86	75.03± 3.62*	0.001	74.80 ± 3.50*	0.001
Height	155.70 ± 2.99	154.43 ± 2.90	0.204	154.73 ± 2.66	0.394
BMI kg/m^2	25.10 ± 1.01	30.59 ± 1.10*	0.001	31.21 ± 1.12*	0.001
Fasting Blood Glucose mg/dl	90.48 ± 10.46	149.03 ± 21.25*	0.001	155.60 ± 19.18*	0.001
Cholesterol mg/dl	130.20 ± 23.70	253.10 ± 18.64*	0.001	261.0 ± 21.21*	0.001
Triglycerides mg/dl	91.01 ± 13.70	167.00 ± 20.74*	0.001	180.03 ± 14.08*	0.001
HDL Cholesterol mg/dl	46.65 ± 7.48	41.02 ± 4.16*	0.001	37.86 ± 4.02*	0.001
LDL Cholesterol mg/dl	65.41 ± 22.56	169.07 ± 26.07*	0.001	188.20 ± 27.35*	0.001
VLDL Cholesterol mg/dl	18.10 ± 2.74	33.00 ± 4.22*	0.001	36.00 ± 2.85*	0.001
Nitric oxide µmol/l	92.30 ± 4.97	89.31 ± 3.06	0.099	50.89 ± 5.44*	0.001
Base Line Diameter (mm)	3.893 ± 0.46	3.79 ± 0.74	0.856	3.59 ± 0.58	0.161
Base Line Flow (ml/min)	130.4 ± 80.4	132 ± 69.8	0.977	119.9±53.5	0.486
Reactive Hyperemia Flow (ml/min)	490 ± 263.2	432±180.5	0.608	401.34±214.7	0.021
Flow Mediated vasodilatation %	7.1 ± 2.7	5.49± 2.09	0.05	4.1±1.82*	0.001

**p< 0.001*

Table 2. Comparison between diabetic normouricemic and diabetic hyperuricemic group Tukey's POST-HOC test

Clinical Parameter	Diabetic Normouricemic (Group II A) (n =30)	Diabetic Hyperuricemic (Group II B) (n =30)	P value
	Mean ± SD	Mean ± SD	
Age in yrs	50.80±4.97	51.80± 6.30	0.740
Weight (kg)	75.03± 3.62	74.80 ± 3.50	0.961
Height	154.43 ± 2.90	154.73 ± 2.66	0.913
BMI kg/m^2	30.59 ± 1.1	31.21 ± 1.12	0.768
Fasting Blood Glucose mg/dl	149.03 ± 21.25	155.60 ± 19.18	0.205
Cholesterol mg/dl	253.10 ± 18.64	261.0 ± 21.21	0.310
Triglycerides mg/dl	167.00 ± 20.74	180.03 ± 14.08	0.01
HDL Cholesterol mg/dl	41.02 ± 4.16	37.86 ± 4.02	0.002
LDL Cholesterol mg/dl	169.07 ± 26.07	188.20 ± 27.35	0.012
VLDL Cholesterol mg/dl	33.00 ± 4.22	36.00 ± 2.85	0.005
Nitric oxide µmol/l	89.31 ± 3.06	50.89 ± 5.44*	0.001
Uric acid mg/dl	4.2 ± 0.74	8.30 ± 1.54*	0.001
Base Line Diameter (mm)	3.79 ± 0.74	3.59 ± 0.58	0.391
Base Line Flow (ml/min)	132 ± 69.8	119.9±53.5	0.371
Reactive Hyperemia Flow (ml/min)	432±180.5	401.34±214.7	0.184
Flow Mediated vasodilatation %	5.49± 2.09	4.1±1.82	0.01

**p< 0.001*

5. DISCUSSION

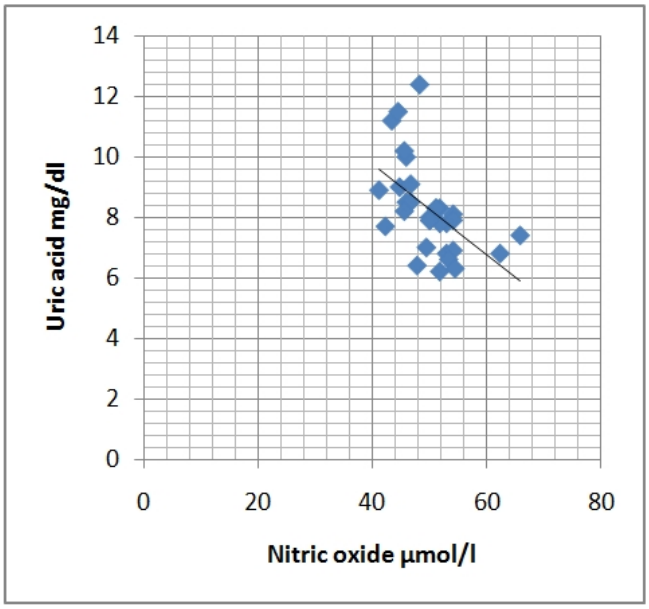
Elevated levels of serum uric acid in type 2 diabetes may be attributed to hyperinsulinemia in the early stages of the disease. Insulin decreases urinary uric acid excretion by enhancing renal urate reabsorption (either through the stimulation of the urate–anion exchanger urate transporter-1 (URAT1) or through the sodium-dependent anion co-transporter in brush-border membranes of the renal proximal tubule [10]. There has been variety of reports on the prevalence of HUA in patients with type 2 diabetes [11-15]. Fang et al. described that the serum uric acid level was higher in diabetic women than in non-diabetic women, although there was no significant difference between diabetic and non-diabetic men [11]. It was reported that HUA was more common in patients with impaired glucose tolerance than in those with diabetes mellitus or normal subjects [12-14]. On the other hand, Alderman et al. reported that the serum uric acid concentration was not associated with the presence of diabetes mellitus [15] and Wen et al. described that it was negatively correlated with the blood glucose level [16]. Li et al. also reported inverse correlations of blood glucose levels with serum uric acid concentration in patients with type 2 diabetes mellitus [17].

In this study, HUA appears to be associated with decreased serum NO levels (Graph 1). The enzymatic reaction of eNOS is of utmost importance to the normal functioning of the

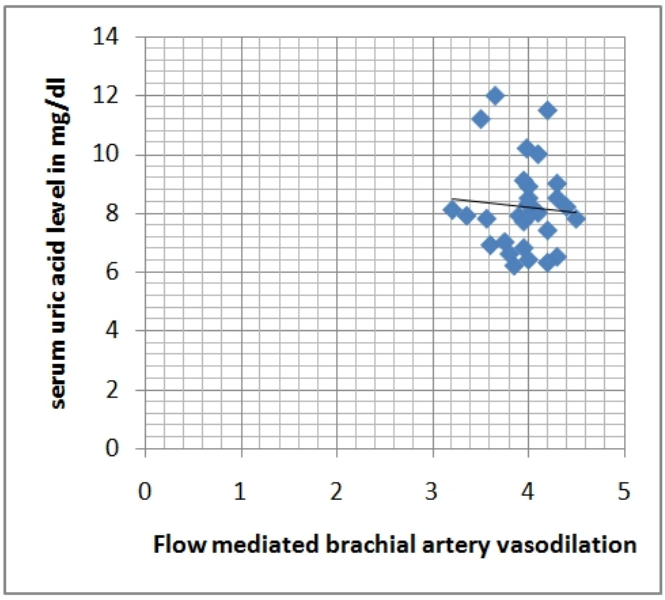
endothelial cell and the intimal interstitium. When this enzyme system uncouples, the endothelium becomes a net producer of superoxide and reactive oxygen species instead of the usual production of the protective antioxidant, NO, thus making uric acid a pro-oxidant. There are multiple causes for endothelial uncoupling of eNOS enzyme, but primary causes are HUA and the antioxidant–pro-oxidant-urate redox shuttle. This shuttle seems to rely heavily on many factors such as timing (early or late disease process), location of the tissue, its pH, the surrounding oxidant milieu and depletion of other local antioxidants. In addition to HUA, glucotoxicity also places an additional redox stress on the arterial vessel wall and capillary endothelium. This in turn also contributes to the switch of uric acid from antioxidant to pro-oxidant state, leading to endothelial dysfunction [18].

In most cells, glucose is metabolized to sorbitol and fructose by aldose reductase and sorbitol dehydrogenase, leading to alteration in NADH/NAD and NADPH/NADP ratios respectively.[19,20] This redox imbalance favours the accumulation of triose phosphates which increases the formation of methylglyoxal and advanced glycation end products (AGEs). This enhances oxidative stress and also contributes to hyperlipidemia. In this present study hyperlipidemia was noted to be a common finding in both the groups of type 2 diabetes mellitus irrespective of hyperuricemia (Table 1)

FMD measurements in this study demonstrate that HUA may be a cause of endothelial dysfunction via decreased NO production. Graph 2 depicts an inverse correlation between serum NO and FMD. Furthermore, Table 1 and Fig. 1. demonstrate reduced brachial artery dilatation in type 2 diabetes mellitus with HUA as compared to controls (Fig. 2.).



Graph 1. Correlation between serum uric acid and nitric oxide level in group II B



Graph 2. Correlation between serum uric acid and flow mediated brachial artery vasodilatation in group II B

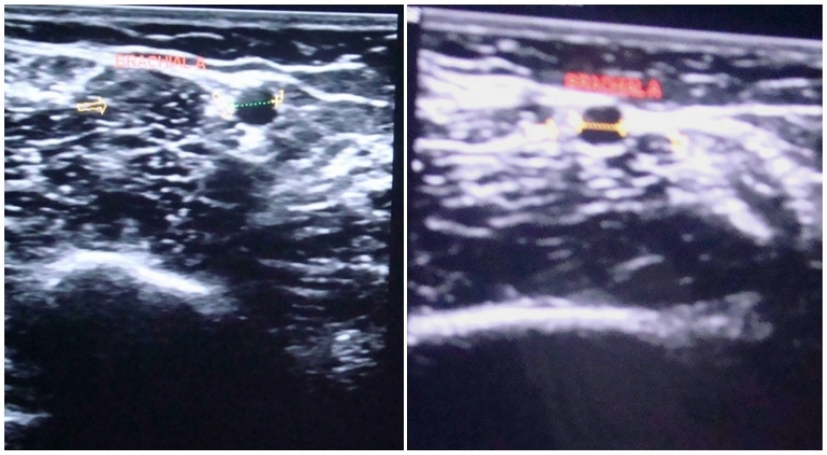


Fig. 1. Flow mediated brachial artery vasodilatation measurement in a diabetic hyperuricemic patient

Before dilatation 3.5 X 10^{-3} micrometer, After dilatation 3.8 X 10^{-3} micrometer

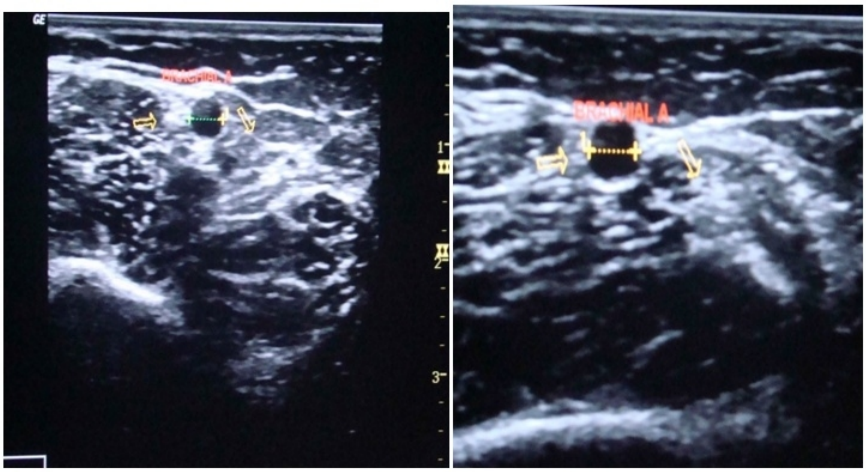


Fig. 2. Flow mediated brachial artery vasodilatation measurement in a healthy control

Before dilatation3.7 X 10^{-3}micrometer, After dilatation 4.4 X 10^{-3} micrometer

5. CONCLUSION

As demonstrated in this study, endothelial dysfunction is common in both subgroups of patients with type 2 diabetes mellitus with a definite trend of correlation to decreased FMD. Hyperuricemia which may be one of the factors in endothelial dysfunction is associated with uncoupling of eNOS enzyme via decreased NO production in patients with type 2 diabetes mellitus. Since the endothelium provides the first line of physiological defense against atherosclerosis, endothelial health is of utmost importance to the prevention of cardiovascular events. The reversal of HUA may play a role in alleviating the development of angiopathy. And as such, monitoring serum uric acid levels in type 2 diabetes patients may prove useful to clinicians. The use of uric acid lowering treatment in hyperuricemic diabetic patients may also improve endothelial function, but this remains to be demonstrated.

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CONSENT

Authors declare that written informed consent was obtained from the patients before their participation in the study.

ETHICAL APPROVAL

All authors hereby declare that approval from Institutional ethics review committee was obtained and study was carried out as per standards.

COMPETING INTERESTS

The authors have no competing interests.

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