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Asymmetric dimethylarginine levels in patients with diabetic nephropathy

Diabetic nephropathy is a major cause of end-stage renal disease worldwide and is characterised by persistent albuminuria, functional and structural changes in the glomerulus. Patients with diabetes mellitus with kidney damage are at higher risk of fatal and nonfatal cardiovascular events.[1] Evidence shows that nitric oxide pathway plays an important role in the development of vascular complication in diabetes. Nitric oxide levels are decreased in diabetic nephropathy due to increased asymmetric dimethyl arginine (ADMA) which is a competitive inhibitor of nitric oxide synthase.[2] Microalbuminuria is the first detectable sign of renal dysfunction in diabetic patients. Both ADMA and microalbuminuria represent markers of endothelial dysfunction which increases the cardiovascular risk in these patients. Therefore this study was designed to study asymmetric dimethyl arginine levels in patients with diabetic nephropathy and its association with proteinuria.

For this 20 patients diagnosed to have diabetic nephropathy defined as microalbuminuria of 30 to 300 mg albumin/24 hours or albumin to creatinine ratio (ACR) of 3.4-34.0 mg/mmol (30-300 mg/g) on two occasions[3] attending the Nephrology and Endocrinology outpatient department of Sri Venkateswara Institute of Medical Sciences, Tirupati were recruited as cases. Twenty, age-and gender-matched healthy individuals from among the hospital staff were taken as controls. The data of family history and personal history was collected through standard questionnaire. Patients with macrovascular complications such as cardiovascular, cerebrovascular, and peripheral vascular diseases were excluded from the study. Five mL of peripheral venous blood were collected in heparinized vial after 12 hours of fasting for the estimation of nitrate and ADMA. Plasma was centrifuged at 2000 rpm for 15 min, separated and stored at –80 ^{0}C until analysis. Spot urine samples were collected and processed for the estimation of creatinine and microalbumin (MA).

Plasma nitrates were estimated by cadmium reduction method[4], plasma ADMA levels was estimated by high performance liquid chromatography[5] and microalbumin was estimated by turbidimetry method using Beckman CX5 fully automated analyzer (Beckman, CA, USA). All the values obtained were expressed as mean ± standard error of mean (SEM). Mann-Whitney 'U' test was used to compare the difference in the parameters studied. Nitric oxide levels measured as plasma nitrates were found to be significantly lower in patients with diabetic nephropathy when compared to controls (Table 1). This can be due to the increased levels of plasma ADMA. The present study also found significantly (Table 1) higher levels of ADMA in diabetic nephropathy patients compared to controls. ADMA, a naturally occurring L-arginine metabolite and a competitive inhibitor of endothelial nitric oxide synthase (eNOS), which reduces the production of nitric oxide.[6] The kidney has a central role in ADMA

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Table 1: Biochemical parameters studied in study and control groups

Parameter	Control group* (n=20)	Study group* (n=20)	p-value
ADMA (μmol/L)	0.76 ± 0.09	2.1 ± 0.5	0.047
NO (μmol/L)	37.9 ± 1.4	28.6 ± 1.8	0.001
MA (mg/dL)	0.45±0.084	6.86 ± 2.33	0.043

* data presented as Mean ± Standard error of Mean (SEM)

ADMA = asymmetric dimethyl arginine; NO = nitric oxide; MA = microalbumin

metabolism. Approximately 20% of ADMA is excreted in urine. Therefore, decreased GFR may also be responsible for the elevation of plasma ADMA levels thereby inhibiting NO generation. NO as the endothelium-derived relaxing factor (EDRF) is the most important for the maintenance of vascular homeostasis by inhibition of platelet aggregation, leukocyte migration, cellular adhesion, and vascular smooth muscle cell proliferation.[7] A decreased synthesis or decreased bioavailability of nitric oxide, leads to an impairment of endothelium dependent relaxations (EDR), characterized by endothelial dysfunction. Reduced eNOS-derived NO bioactivity, is the critical step for atherogenesis. Decreased nitric oxide in diabetes contributes to the progression of kidney disease and also involved in the cardiovascular complications.[6]

Urinary MA levels were found to be significantly (Table 1) higher in the study group compared to controls. Proteinuria is a state of increased protein turnover and hence when proteins undergo proteolysis, free methylarginines (ADMA and SDMA) are released as recently described for states of increased protein metabolism and insulin resistance[8]. Kidney plays an important role since it is the route for clearance of ADMA as well as helps in its degradation by the enzyme dimethyl arginine dimethylaminohydrolase (DDAH), which is abundantly expressed in renal endothelial and tubule cells. It can be hypothesized that protein overload alters the expression of dimethyl arginine dimethylaminohydrolase (DDAH) thereby favoring ADMA accumulation.[9] In present study also the plasma levels of ADMA were found to be significantly (Table 1) higher in patients compared to controls. Spearman's correlation analysis was used to assess the correlation between renal dysfunction markers such as creatinine and microalbumin. Serum creatinine levels showed a significant positive correlation with ADMA ($\rho = 0.543$, $p=0.030$) and urinary-MA ($\rho= 0.584$, $p=0.014$). However, no correlation was found between ADMA and MA ($\rho=0.052$, $p=0.854$).

Therefore the findings of the present study suggest that there is an increase in plasma ADMA levels in patients with diabetic nephropathy compared to controls. This can lead to decreased nitric oxide levels due to inhibition of eNOS by ADMA in patients with diabetic nephropathy. The decreased nitric oxide levels are not only involved in renal tubular damage causing renal dysfunction but also cause endothelial dysfunction. Also, the increased microalbuminuria observed in these patients further adds to the cardiovascular risk in these patients at the early stages itself.

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