Synergistic Association of Metabolic Syndrome and Overt Nephropathy With Elevated Asymmetric Dimethylarginine in Serum and Impaired Cutaneous Microvasodilation in Patients With Type 2 Diabetes

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Chronic kidney disease (CKD) is a major risk factor for cardiovascular disease as well as end-stage renal disease (1). Diabetic nephropathy is an important cause and exacerbating factor in CKD. Recent studies have demonstrated a close relationship between prevalence of the metabolic syndrome and CKD (2,3). Microvascular endothelial dysfunction resulting from impaired nitric oxide biosynthesis and bioavailability is associated with occurrence of metabolic syndrome and type 2 diabetes (4,5). de Jongh et al. (6) found cutaneous microvascular function to be related to degree of insulin resistance in obese women, suggesting that microvascular endothelial dysfunction in the skin can predict metabolic syndrome–related microangiopathy and insulin resistance. Thus, microvascular endothelial dysfunction may be a causal factor linking metabolic syndrome and CKD.

Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, causes vasoconstriction and limits blood flow, resulting in elevated blood pressure in humans (7,8). Elevated plasma ADMA concentrations are found in patients with renal insufficiency (9), diabetes (10), and metabolic syndrome (11). Accordingly, increased plasma ADMA may contribute to microvascular endothelial dysfunction observed in individuals with metabolic syndrome or with CKD.

We examined cutaneous microvascular responses to heat applied locally to the skin of the dorsum of the foot in type 2 diabetic patients with or without metabolic syndrome or overt nephropathy, measuring cutaneous blood flow by laser Doppler flowmetry as well as serum ADMA concentration.

RESEARCH DESIGN AND METHODS

We studied 105 type 2 diabetic patients (41 female and 64 male). We excluded patients who had any known CKD before diagnosis with diabetes.

Diagnosis of metabolic syndrome was based on National Cholesterol Education Panel–Adult Treatment Panel III criteria (12). We used a BMI cutoff value >25.0 kg/m^2 to define obesity (13), since waist circumference is unsuitable for detection of obesity in a Japanese population.

Overt nephropathy was defined as clinical proteinuria and/or a creatinine clearance <60 ml/min. We defined clinical proteinuria as urinary albumin excretion >300 mg/24 h. As an index of glomerular filtration rate, creatinine clearance was calculated using the same 24-h urine collection.

On the basis of the two definitions above, we divided the diabetic patients into four groups: group A, those with no metabolic syndrome and no overt nephropathy (n = 38); group B, those with metabolic syndrome but no overt nephropathy (n = 32); group C, those with no metabolic syndrome but with overt nephropathy (n = 16); and group D, those with both metabolic syndrome and overt nephropathy (n = 19). Cardiovascular disease was defined as coronary artery disease, stroke, and/or peripheral vascular disease.

Skin blood flow measurements

Cutaneous blood flow (flux) was recorded on the dorsum of the right foot using laser Doppler flowmetry (Perimed PF4; Perimed, Stockholm, Sweden). A thermostatic laser Doppler probe, which heats the skin locally, was placed on the dorsum of the right foot. Local temperature was increased gradually from 32°C to 44°C (14). Change in skin blood flow (Δ flux) was defined as: peak flux at 44°C (in perfusion units [PU]) − basal flux (in PU).

The ankle-brachial index was determined as the ratio of ankle systolic blood pressure to brachial systolic blood pressure (Colin Medical Technology, Komaki, Japan).

Serum concentrations of ADMA were measured by high-performance liquid chromatography. Plasma thrombomodulin, a marker of endothelial injury (15), was measured by an enzyme immunoassay (Fujirebio, Tokyo, Japan).

Data are presented as the mean ± SD or the median (interquartile range), unless otherwise indicated. Differences were assessed by ANOVA or the Kruskal-Wallis test. Significance of differences in prevalence between groups was analyzed by a χ^2 test. A P value <0.05 was accepted as indicating statistical significance.

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Abbreviations: ADMA, asymmetric dimethylarginine; CKD, chronic kidney disease.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Patient characteristics and laboratory data in diabetic groups categorized according to the presence of metabolic syndrome and overt nephropathy*

Variable	Group A: no metabolic syndrome/ no overt nephropathy	Group B: metabolic syndrome/ no overt nephropathy	Group C: no metabolic syndrome/ overt nephropathy	Group D: metabolic syndrome/ overt nephropathy
n (female/male)	38 (14/24)	32 (18/14)	16 (6/10)	19 (4/15)
Age (years)	62.6 ± 9.7	56.8 ± 13.5	62.6 ± 10.8	60.5 ± 10.8
BMI (kg/m^2)	21.6 ± 2.6	27.6 ± 5.6‡	22.9 ± 3.5§	26.2 ± 2.7‡¶
Diabetes duration (years)	12.6 ± 9.0	9.9 ± 7.1	11.9 ± 7.2	12.3 ± 7.9
Fasting plasma glucose (mmol/l)	9.8 ± 2.8	9.6 ± 3.3	9.8 ± 3.5	9.8 ± 3.2
HbA_{1c} (%)	10.2 ± 2.0	9.5 ± 1.8	9.3 ± 1.7	9.6 ± 1.6
Total cholesterol (mmol/l)	4.87 ± 0.83	5.22 ± 0.73	5.39 ± 1.36	5.29 ± 0.80
Triglyeride (mmol/l)	1.54 ± 0.51	2.91 ± 1.40‡	1.57 ± 0.51§	2.94 ± 0.96†#
HDL cholesterol (mmol/l)	1.40 ± 0.37	1.03 ± 0.24‡	1.27 ± 0.32‡	1.10 ± 0.26†
Cretinine clearance (ml/min)	82.1 ± 24.8	96.0 ± 47.4	68.7 ± 30.1†§	58.8 ± 31.4†¶
Urinary albumin excretion (mg/24h)	15.0 (11.0–29.5)	46.5 (13.5–84.0)	338 (79.5–664)‡§	386 (331–766)†#
Thrombomodulin (FU/ml)	2.55 (2.20–3.00)	2.70 (2.20–3.00)	3.40 (2.75–4.00)*	3.70 (3.05–4.70)‡‖
Serum ADMA (μmol/l)	0.47 ± 0.08	0.51 ± 0.08*	0.53 ± 0.09*	0.55 ± 0.10†
Ankle-brachial index	1.14 ± 0.06	1.11 ± 0.16	1.12 ± 0.15	1.16 ± 0.17
Basal flux (PU)	11.8 (6.6–16.4)	10.8 (7.8–21.5)	11.4 (8.4–16.5)	10.6 (7.4–14.6)
Peak flux (PU)	43.1 (32.0–59.6)	34.9 (25.3–43.8)	56.3 (33.4–79.2)	28.4 (20.1–31.5)*¶
Δ flux (PU)	29.9 (22.6–47.5)	17.7 (13.3–36.4)	41.9 (16.9–68.7)	15.7 (9.3–18.9)*¶
Hypertension (*n* [%])	6 (15.8)	22 (68.8)‡	4 (25.0)#	13 (68.4)‡#
Cardiovascular disease (*n* [%])	5 (13.2)	8 (25.0)	3 (18.8)	7 (36.8)*
Retinopathy (none /background retinopath/proliferative)	22/9/7	19/2/11	5/5/6	5/4/10
Treatment (diet/oral hypoglycemic agents/insulin)	2/26/10	4/25/3	1/12/3	0/15/4

Data are means ± SD or median (interquartile range), unless otherwise indicated. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ vs. group A. §$P < 0.05$, ‖$P < 0.001$ vs. group B. ¶$P < 0.05$, #$P < 0.001$ vs. group C.

RESULTS — Patient characteristics and laboratory data in these subgroups are summarized in Table 1. Plasma thrombomodulin was significantly higher in diabetic patients in group D than in groups A or B ($P < 0.001$, respectively). Serum ADMA was significantly higher in group D ($P < 0.001$), group B ($P < 0.05$), and group C ($P < 0.05$) than in group A. Thus, serum ADMA was highest in diabetic patients who had both metabolic syndrome and overt nephropathy. Cardiovascular disease was more prevalent in group D than in group A ($P < 0.05$).

We evaluated changes in cutaneous blood flow on the dorsum of the right foot in response to local heat in the four subgroups. Basal flux was similar between these groups. Both peak and Δ flux were significantly smaller in group D than in groups A ($P < 0.05$) or C ($P < 0.05$) (Table 1).

Serum ADMA correlated positively with urinary albumin excretion ($r = 0.22$, $P < 0.05$) and thrombomodulin ($r = 0.28$, $P < 0.01$) and negatively with HbA_{1c} ($r = -0.20$, $P < 0.05$), HDL cholesterol ($r = -0.20$, $P < 0.05$), and creatinine clearance ($r = -0.28$, $P < 0.01$). By multivariate analysis, in a model that explained 45.3% of variation of serum ADMA, only HDL cholesterol ($\beta = -0.197$, $P = 0.048$) and creatinine clearance ($\beta = -0.202$, $P = 0.044$) were independent determinants of serum ADMA in patients with type 2 diabetes.

CONCLUSIONS — We found that serum concentrations of ADMA were significantly higher in diabetic patients with metabolic syndrome and overt nephropathy, metabolic syndrome but no overt nephropathy, or overt nephropathy but no metabolic syndrome than in those with no metabolic syndrome and no overt nephropathy. Plasma thrombomodulin also was significantly higher in diabetic patients with metabolic syndrome and overt nephropathy than in those with no metabolic syndrome and no overt nephropathy or overt nephropathy but no metabolic syndrome. Since ADMA is considered a novel contributor to endothelial dysfunction, these results indicate profoundly impaired endothelial dysfunction in diabetic patients with both metabolic syndrome and overt nephropathy.

The present study showed that serum HDL cholesterol and renal function were independent determinants of serum ADMA in type 2 diabetic patients. ADMA is disposed of via two routes: renal clearance and degradation by the enzyme dimethylarginine dimethylamonihydrolase (7,8). Circulating ADMA accumulates in patients with CKD such as diabetic nephropathy. Low HDL cholesterol, a component of metabolic syndrome, was associated with high serum ADMA. Thus, serum ADMA was highest in diabetic patients with both metabolic syndrome and overt nephropathy.

The present study demonstrated for the first time that cutaneous microvasodilation measured in the foot in response to local heat was significantly lower in diabetic patients with both metabolic syndrome and overt nephropathy than in those with no metabolic syndrome and no overt nephropathy or in those with overt nephropathy but no metabolic syndrome. Although cutaneous microvasodilation in response to local heat is a complex re-

sponse involving several factors including the axon reflex and endothelial nitric oxide (16,17), such impaired vasodilation may reflect microvascular endothelial dysfunction. Thus, coexistence of metabolic syndrome and overt nephropathy is synergistically related to profound impairment of cutaneous microvascular function via elevated serum ADMA, increasing cardiovascular risk. However, a major limitation of this study is that we could not definitely exclude all patients with nondiabetic kidney disease completely, since we did not obtain renal biopsy specimens histologic diagnosis. Therefore, we referred to overt nephropathy instead of overt diabetic nephropathy.

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