Original Article

Title: Renal Manifestations of Metabolic Syndrome in Type 2 Diabetes

Short running title: Renal Manifestations of the Metabolic Syndrome

KO HANAI [1,2], TETSUYA BABAZONO [1,2], and YASUHIKO IWAMOTO MD [1]

From [1]the Division of Nephrology and Hypertension and [2]Department of Medicine, Diabetes Center, Tokyo Women's Medical University School of Medicine, Tokyo 162-8666, Japan

Address correspondence and reprint requests to

Tetsuya Babazono, MD, PhD

Division of Nephrology and Hypertension, Diabetes Center, Tokyo Women's Medical University School of Medicine

8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan

Phone: +81-03-3353-8111

Fax: +81-03-3358-1941

E-mail: babazono@dmc.twmu.ac.jp

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Abstract:

Aim: The association between metabolic syndrome (MS) and renal outcomes in diabetic patients remains unclear. We conducted a cross-sectional study to assess the association between MS and albuminuria and decreased glomerular filtration rate (GFR) in type 2 diabetic patients.

Methods: We studied a total of 1003 adult Japanese patients with type 2 diabetes, 582 men, with a mean (± SD) age of 62 ± 12 years. Patients with macroalbuminuria, defined as a urinary albumin-to-creatinine ratio (ACR) ≥ 300 mg/g Cr, and those with renal insufficiency, defined as serum creatinine ≥ 2.0 mg/dl, were excluded. MS was assessed according to Japanese definition proposed in 2005. Microalbuminuria and decreased GFR were defined as ACR of 30-299 mg/g Cr and estimated GFR < 60 ml/min/1.73 m^2, respectively.

Results: The prevalence of microalbuminuria was significantly higher in patients with MS than those without. In contrast, the prevalence of decreased GFR was comparable between patients with and without MS. Waist circumference was selected as a significant variable in the logistic regression analysis for microalbuminuria.

Conclusion: Microalbuminuria, but not decreased GFR, is independently associated with MS and solely with increased waist circumference in Japanese patients with type 2 diabetes.

Keywords: metabolic syndrome, microalbuminuria, decreased GFR

(199 words)

INTRODUCTION

Metabolic syndrome is characterized by a clustering of cardiovascular risk factors, including central obesity, hypertension, dyslipidemia, and fasting hyperglycemia [1]. Recently, a potential association between metabolic syndrome and predisposition to microalbuminuria and chronic kidney disease (CKD) has been proposed. A higher prevalence of microalbuminuria was demonstrated in both non-diabetic and diabetic individuals with metabolic syndrome [2-8], and prevalence of CKD, defined as an estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73 m^2, was found to be higher in adults with than those without metabolic syndrome [6,9,10]. In diabetic patients, the association between metabolic syndrome and these renal outcomes may be masked by the presence of poor glycemic control, which is a prognostic marker for both microalbuminuria and diabetic nephropathy. In addition, hypertension and lipid abnormalities are known to be strong risk factors for the development of diabetic nephropathy [4,11]. Therefore, earlier observations demonstrating a relationship between metabolic syndrome and microalbuminuria in diabetic patients may be simply explained by the presence of hyperglycemia, hypertension, and dyslipidemia. Currently, no data are available to assess the relationship between metabolic syndrome and decreased GFR in this population.

Abdominal obesity has been recently acknowledged as the fundamental component of metabolic syndrome [12,13]. A worldwide consensus definition of metabolic syndrome, recently established by the International Diabetes Federation (IDF), requires elevated waist circumference for the diagnosis of the syndrome [12]. Obesity, especially abdominal obesity, has been associated with higher cardiovascular mortality and renal outcomes, microalbuminuria and CKD, in non-diabetic and diabetic individuals; however, information is scarce regarding whether and to what extent abdominal obesity affects renal outcomes in diabetic

patients [11]. We, therefore, conducted this cross-sectional study to examine whether components of metabolic syndrome, particularly abdominal obesity, predispose Japanese adults with type 2 diabetes to microalbuminuria and/or decreased GFR.

MATERIALS AND METHODS

Study subjects and protocol

This was a hospital-based cross-sectional study of 1003 Japanese patients with type 2 diabetes. Adult type 2 diabetic patients were recruited from ambulatory and hospitalized patients presenting at the Diabetes Center, Tokyo Women's Medical University Hospital, in Tokyo, Japan, during the period of April 1, 2005 through March 31, 2007. Type 2 diabetes was diagnosed according to the Japan Diabetes Association criteria [14].

At a regular ambulatory visit or on the first day of hospitalization, subjects underwent an anthropometric and physical examination, including blood pressure, height, weight, and waist circumference. Laboratory examinations included plasma glucose, hemoglobin A_{1C} (A1C), serum lipids, serum creatinine (using fasting blood samples), and urinary albumin, measured in the first morning urine specimen. Patients with clinical albuminuria, defined as a urinary albumin-to-creatinine ratio (ACR) ≥ 300 mg/g Cr, and those with renal insufficiency, defined as serum creatinine ≥ 2.0 mg/dl, were excluded. Patients who had malignant disease, severe liver dysfunction, thyroid disease, or who had undergone lower limb amputation were also excluded.

Definition of the metabolic syndrome

Metabolic syndrome was assessed according to the Japanese Society for Internal Medicine definition proposed in 2005 [13]. In this definition, subjects must

have central obesity, defined as waist circumference ≥ 85 cm for men and ≥ 90 cm for women, plus at least two of the following three factors: atherogenic dyslipidemia (triglyceride ≥ 150 mg/dl and/or HDL cholesterol level < 40 mg/dl, or specific treatment for these lipid abnormalities); elevated blood pressure (systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg, or treatment of previously diagnosed hypertension); or increased fasting plasma glucose (≥ 110 mg/dl). Because all of the subjects included in this study were diabetic (and by definition had elevated fasting plasma glucose), central obesity plus either elevated blood pressure or dyslipidemia, or both, was sufficient for diagnosis of metabolic syndrome.

Outcome measurements

We focused on the relationship between metabolic syndrome and two renal outcome measures, microalbuminuria and decreased eGFR. Urinary albumin was measured using the latex agglutination method and normalized using urinary creatinine. Normo- and microalbuminuria were defined as ACR less than 30 mg/g Cr and 30-299 mg/g Cr, respectively [15]. GFR was estimated using the following equation, originating from the Modification of Diet in Renal Disease (MDRD) Study group [16], and refitted for Japanese individuals, as recently proposed by the Working Group of Japan Chronic Kidney Disease Initiative (JCKDI): eGFR = 186 x $SCr^{-1.154}$ x $Age^{-0.203}$ x 0.742 (if female) x 0.881, where SCr = serum creatinine [17]. Serum creatinine was measured by an enzymatic method and the value was calibrated, using the following equation, prior to inclusion in the equation for estimation of GFR: serum creatinine (Jaffe's method) = 1.0302 x serum creatinine (enzymatic method) + 0.2648 [$r=0.999$, $p<0.001$]. Decreased eGFR was defined as an eGFR less than 60 ml/min/1.73 m^2, irrespective of the presence of microalbuminuria.

Other biochemical analysis

Plasma glucose, total cholesterol and triglycerides were measured by enzymatic methods. HDL cholesterol was measured using polyethylene-glycol-pretreated enzymes. LDL cholesterol was determined directly by a homogeneous enzymatic assay. A1C was measured by HPLC. Plasma insulin was measured (only in diabetic patients without insulin therapy) by electrochemiluminescence immunoassay (ECLIA). The homeostasis model assessment (HOMA IR: fasting plasma glucose [mg/dl] x fasting insulin [μU/ml]/405) was used to derive insulin sensitivity.

Statistical analysis

Data were expressed as percentage, arithmetic mean ± SD or geometric mean with 95% confidence interval (CI), as appropriate according to data distribution. For statistical analyses, Student t test, Fisher's exact probability test, Steel's multiple comparison test, one-way ANOVA, analysis of covariance (ANCOVA), simple Pearson's correlational analysis, multiple regression analysis, and logistic regression analysis were used appropriately according to the situation. All statistical analyses were performed using the SAS version 9.13 (SAS Institute, Cary, NC). P values less than 0.05 were considered significant.

RESULTS

Patient characteristics

We studied a total of 1003 adults with type 2 diabetes, 421 women and 582 men, with a mean (± SD) age of 62 ± 12 years. There were 242 patients treated with insulin, 521 patients treated with oral hypoglycemic drugs and 240 patients without

any anti-diabetic medication. At the time of evaluation, 300 patients (29.9%) had microalbuminuria.

Among the 1003 subjects studied, 592 (59.0%) met the criteria for metabolic syndrome proposed by the Japanese Society for Internal Medicine. Demographic characteristics and laboratory data of the subjects with and without metabolic syndrome are presented in Table 1. Patients with metabolic syndrome were younger, more likely to be men, and more likely to be treated with angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) than those without metabolic syndrome. The proportions of patients with duration of diabetes ≥ 10 years and the prevalence of diabetic retinopathy were comparable between the two groups.

As expected, patients with metabolic syndrome had larger waist circumference, higher body mass index (BMI), blood pressure, serum triglyceride, and LDL cholesterol, and lower HDL cholesterol levels than those without. There was no significant difference in A1C between the two groups (Table 1).

Relationship with microalbuminuria and decreased eGFR

The prevalence of microalbuminuria and geometric mean (95% CI) urinary ACR were significantly higher in patients with metabolic syndrome than those without the syndrome (Table 2). According to logistic regression analysis, concurrent metabolic syndrome was associated with a three-fold greater prevalence of microalbuminuria. There were also significant graded relationships between the number of components and the prevalence of and adjusted odds ratio for microalbuminuria or geometric mean ACR. In contrast, neither prevalence of decreased eGFR, adjusted odds ratio for decreased eGFR, nor adjusted mean GFR were significantly different between diabetic patients with and without metabolic

syndrome. The presence of one, two or three components of metabolic syndrome was associated with a significantly higher risk for decreased eGFR relative to patients with no component.

In univariate correlational analyses, waist circumference correlated positively with logarithmically transformed urinary ACR ($r=0.179$, $p<0.001$, Fig. 1-A) but not with eGFR ($r=0.043$, $p=0.170$, Fig. 1-B). To determine independent effects of waist circumference on renal outcomes, multiple regression analysis and logistic regression analysis were conducted. In both analyses, the components of metabolic syndrome treated as continuous variables were incorporated into the model, as well as other covariates including age, sex, presence of diabetic retinopathy, use of ACE inhibitors and ARBs, A1C, and LDL cholesterol. Waist circumference was selected as a significant variable in the multiple regression analysis for ACR (Table 3) and in the logistic regression analysis for microalbuminuria (Table 4). In either the multivariate regression analysis or logistic regression analysis, waist circumference was not selected as variable significantly associated with for eGFR (Table 3,4).

Discussion

In this hospital-based cross-sectional study, we demonstrated that microalbuminuria was closely associated with metabolic syndrome in Japanese type 2 diabetic patients. The prevalence of microalbuminuria increased progressively with increase in the number of components of metabolic syndrome. In addition, we demonstrated that larger waist circumference, the fundamental component of metabolic syndrome, was a strong predictor of microalbuminuria. This relationship was independent of other components of metabolic syndrome that are well-known risk factors for the development of microalbuminuria in diabetic patients. In contrast,

decreased eGFR, defined as an eGFR less than 60 ml/min/1.73 m^2, showed no association with metabolic syndrome or waist circumference.

The mechanisms implicating abdominal obesity in the pathogenesis of microalbuminuria remain unknown. However, enhanced production of adipocytokines by abdominal fat, increased activity of the renin-angiotensin-aldosterone system, and insulin resistance, all of which are closely linked to abdominal obesity, may explain the relationship [18-22]. Elevated levels of serum leptin may contribute to renal impairment via glomerular hyperfiltration by activating sympathetic nervous system, or direct injury to glomerular endothelial and mesangial cells [23,24]. Increased plasma aldosterone levels, which have been recently found to be associated with metabolic syndrome and independently with increased waist circumference, may link abdominal obesity to microalbuminuria by aldosterone-induced renal injury [25]. Cross-sectional studies have demonstrated that hyperinsulinemia, as a manifestation of insulin resistance, is related to microalbuminuria when accompanied by essential hypertension [20,22].

In contrast to microalbuminuria, decreased GFR, another renal manifestation of type 2 diabetes, was not significantly associated with metabolic syndrome or abdominal obesity in this study. This finding is inconsistent with previous studies in non-diabetic individuals, which indicate an association between metabolic syndrome and increased waist circumference and decreased GFR [6,9,10]. However, Retnakaran et al. demonstrated that decreased waist circumference was associated with decreased GFR in type 2 diabetic patients [11]. Although the reasons for this discrepancy are unclear, this may suggest different mechanisms of renal insufficiency between diabetic and non-diabetic patients. Furthermore, the cross-sectional design of this study may have limited its ability to assess such an association, since GFR in diabetic patients may be affected by other factors than in

non-diabetic subjects. In the pathogenesis of obesity-associated glomerulopahty, hyperfiltration is of fundamental importance. In addition, hyperglycemia *per se* has been well known to be associated with hyperfiltration in the early stages of diabetic nephropathy, and GFR starts to decrease thereafter. Treatment with ACE inhibitors and ARBs, the first-line therapies for hypertension in diabetic patients, may also affect GFR. Further longituidial studies are, thus, needed to determine whether abdominal obesity affects change in GFR in diabetic patients.

Our study has several limitations. First, this was a cross-sectional study; therefore close associations between microalbuminuria and metabolic syndrome/ increased waist circumference in this study do not necessarily indicate that albuminuria increases in relation to waist circumference. To ascertain this, longitudinal study will be needed. Secondly, microalbuminuria was determined from a single measurement of urinary ACR possibly leading to improper categorization because of marked day-to-day variability in albumin excretion. Although we did not obtain multiple measurements of urinary ACR, we restricted the timing of urine collection to the first morning to minimize exercise-induced and diurnal variations [26]. Thirdly, this study was carried out in an urban university hospital in an ethnically homogenous population, which may not be representative of the entire type 2 diabetic patient population. The generalizability of these findings will need to be confirmed in future studies. Finally, the performance of the estimation for GFR remains controversial.

In conclusion, this hospital-based cross-sectional study demonstrated that microalbuminuria in patients with type 2 diabetes is closely associated with metabolic syndrome, and, in particular, with its fundamental component waist circumference. Decreased GFR dose not appear to show similar relationship. A cause-effect relationship between abdominal obesity and renal outcomes remains to

be clarified in longitudinal studies.

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Figure legend

Figure. Correlational analysis between waist circumference and urinary albumin-to-creatinine ratio (ACR, panel A) and estimated glomerular filtration rate (eGFR, panel B) in 1003 diabetic patients

Table 1. Demographic and laboratory data in diabetic patients with and without the metabolic syndrome

	Metabolic syndrome		P value
	Absent (N=411)	Present (N=592)	
Age (years)	63 ± 11	60 ± 13	<0.001
Men (%)	45.5	66.7	<0.001
Diabetes duration ≥ 10 years (%)	54.3	52.2	0.560
Diabetic retinopathy (%)	44.3	43.7	0.895
Medication for diabetes, none/oral/insulin (%)	25/50/25	23/54/23	0.548
ACE inhibitors (%)	9.8	15.0	0.016
ARBs (%)	24.2	35.0	<0.001
History of coronary artery disease (%)	8.8	15.7	0.002
History of cerebrovascular disease (%)	14.3	16.7	0.327
Body mass index (kg/m^2)	22.9 ± 3.9	27.7 ± 4.1	<0.001
Waist circumference (cm)	81.3 ± 8.0	96.6 ± 8.9	<0.001
Systolic blood pressure (mmHg)	129 ± 18	134 ± 17	<0.001
Diastolic blood pressure (mmHg)	73 ± 11	78 ± 12	<0.001
Laboratory data			
Glucose (mg/dl)	150 ± 44	162 ± 48	<0.001
Insulin (μU/ml)	4.5 (4.2-4.9)	7.4 (6.9-7.8)	<0.001
HOMA-IR	1.61 (1.47-1.77)	2.86 (2.68-3.04)	<0.001
A1C (%)	8.5 ± 2.0	8.7 ± 1.9	0.160
Triglyceride (mg/dl)	105 (100-110)	141 (135-147)	<0.001
HDL cholesterol (mg/dl)	52 ± 14	46 ± 13	<0.001
LDL cholesterol (mg/dl)	117 ± 31	122 ± 32	0.015

Abbreviations: ACE: angiotensin-converting enzyme, ARB: angiotensin-receptor blocker, HOMA-IR: homeostasis model assessment of insulin resistance,
A1C: hemoglobin A1C, HDL: high density lipoprotein, LDL: low density lipoprotein,
Data are mean ± SD or geometric mean (95% CI).

Table 2. Comparison of renal parameters in diabetic patients with and without the metabolic syndrome and those classified according to the number of metabolic syndrome components other than hyperglycemia

	Metabolic syndrome		Number of components of metabolic syndrome			
	Absent (N=411)	Present (N=592)	0 (N=73)	1 (N=193)	2 (N=379)	3 (N=358)
Microalbuminuria						
Prevalence (%)	19.2	37.3*	6.9	18.7**	30.1**	40.5**
Adjusted odds ratio (95% CI)	1.00	2.68 (1.94-3.71)*	1.00	2.75 (1.00-7.55)**	5.54 (2.12-14.44)**	8.53 (3.28-22.19)**
Urinary ACR, geometric mean (95% CI [mg/g Cr])						
Crude	14.2 (12.8-15.8)	21.4 (19.6-23.4)*	10.0 (7.8-12.9)	13.7 (11.7-16.0)**	18.3 (16.4-20.5)** †	23.3 (20.8-26.1)** † ‡
Adjusted	14.4 (12.9-15.9)	21.2 (19.5-23.1)*	11.2 (8.7-14.4)	14.0 (12.0-16.4)	18.5 (16.7-20.6)** †	22.2 (19.9-24.8)** † ‡
Decreased eGFR						
Prevalence (%)	24.6	23.8	4.1	21.8**	26.1**	27.4**
Adjusted odds ratio (95% CI)	1.00	1.24 (0.89-1.74)	1.00	5.19 (1.50-17.94)**	6.84 (2.03-23.02)**	8.34 (2.47-28.19)**
Estimated GFR (ml/min/1.73 m^2)						
Mean ± SD	69.7 ± 15.3	70.9 ± 15.3	76.6 ± 14.5	71.5 ± 14.9**	69.7 ± 15.2**	69.3 ± 15.6**
Adjusted mean ± SE	71.0 ± 0.7	69.9 ± 0.6	75.0 ±1.6	72.4 ± 1.0	69.8 ± 0.7** †	69.0 ± 0.7** †

Abbreviations: CI, confidence interval; ACR, albumin-to-creatinine ratio; GFR, glomerular filtration rate. Microalbuminuria and decreased eGFR were defined as albuminuria of 30-299 mg/g Cr and estimated GFR less than 60 ml/min/1.73 m^2, respectively.

Prevalence of microalbuminuria and decreased eGFR was compared using Fisher's exact probability test (between 2 groups) or Steel's multiple comparison test (among 4 groups by the number of metabolic syndrome components).

Adjusted odds ratios for microalbuminuria and decreased eGFR were calculated by multivariate logistic regression analysis with age, sex, presence of diabetic duration ≥ 10 years, presence of diabetic retionopathy, hemoglobin A_{1C}, LDL cholesterol and the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers as covariates.

Adjusted geometric mean urinary ACR and mean eGFR were calculated by the analysis of covariance (ANCOVA) with age, sex, diabetes duration, presence of diabetic retinopathy, HbA_{1C}, LDL cholesterol, and the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers.

* $p<0.05$ vs "Absent", ** $p<0.05$ versus 0 component of metabolic syndrome, † $p<0.05$ versus 1 component of metabolic syndrome, $^{\#}$ $p<0.05$ versus 2 component of metabolic syndrome.

Table 3. Multiple regression analysis with stepwise variable selection to determine potent factors associated with urinary ACR and eGFR in 1003 diabetic patients.

Independent variable	Dependent variable			
	Urinary ACR (mg/g Cr)		eGFR (ml/min/1.73 m^2)	
	Standardized estimate	P value	Standardized estimate	P value
Intercept	0.000	0.006	0.000	<0.001
Age (years)	-		-0.493	<0.001
Sex (men vs women)	-		0.093	<0.001
Waist circumference (cm)	0.144	<0.001	-	
Systolic blood pressure (mmHg)	0.097	0.001	-	
Diabetic duration ≥ 10 years (Yes vs No)	0.083	0.011		
Diabetic retinopathy (Yes vs No)	0.249	<0.001	-	
ACE inhibitors (Yes vs No)	0.076	0.013	-	
ARBs (Yes vs No)	0.073	0.018	-0.115	<0.001
A1C (%)	0.113	<0.001	0.161	<0.001
Triglyceride (mg/dl)	0.103	0.001	-0.122	<0.001
HDL cholesterol (mg/dl)			0.071	0.018
LDL cholesterol (mg/dl)	-		0.054	0.050

Abbreviations: ACR: albumin-to-creatinine ratio, eGFR: estimated glomerular filtration rate, ACE: angiotensin-converting enzyme, ARB: angiotensin receptor blocker, A1C: hemoglobin A1C

Diastolic blood pressure was calculated as other dependent variables than those above, and eGFR and logarithmically ACR were calculated as dependent variables in the analyses for ACR and eGFR, respectively.

Table 4. Logistic regression analysis to determine components of metabolic syndrome with microalbuminuria and decreased eGFR in 1003 diabetic patients

Independent variable	Dependent variable			
	Microalbuminuria		Decreased eGFR	
	Odd ratio (95% CI)	P value	Odd ratio (95% CI)	P value
Large waist circumference (Yes vs No)	2.28 (1.62-3.22)	<0.001	0.99 (0.69-1.41)	0.952
Hypertension (Yes vs No)	2.05 (1.39-3.02)	<0.001	0.86 (0.55-1.36)	0.529
Dyslipidemia (Yes vs No)	1.32 (0.95-1.84)	0.100	2.66 (1.82-3.89)	<0.001

Abbreviations: CI: confidence interval, ACEI: angiotensin-converting enzyme inhibitor ARB: angiotensin receptor blocker,

Odds ratio was adjusted for age, sex, presence of diabetic duration ≥ 10 years, presence of diabetic retinopathy, hemoglobin A1C, LDL cholesterol, usage of ACEI and usage of ARB. Large waist circumference was defined as waist circumference ≥ 85 cm for men and ≥ 90cm for women. Dyslipidemia was defined as triglyceride ≥ 150 mg/dl and/or HDL cholesterol level < 40 mg/dl, or specific treatment for these lipid abnormalities. Hypertension was defined as systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg, or treatment of previously diagnosed hypertension.