Glomerulopathy Associated With Moderate Obesity

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Introduction: Obesity-related glomerulopathy is an established secondary glomerular disease that may occur in obese individuals with a body mass index (BMI) of $\geq$30 kg/m^2. However, patients with moderate obesity (BMI $\leq$ 30 kg/m^2) may also develop this disease.

Methods: A total of 20 patients with grade 1 obesity (25 $\leq$ BMI $<$ 30 kg/m^2) with persistent proteinuria, without evidence of other renal diseases, were analyzed retrospectively. These patients were compared with 20 patients with grade 2 or higher obesity (BMI $\geq$ 30 kg/m^2) with persistent proteinuria. Biopsies of 31 kidney transplant donors as healthy controls were used to compare histologic parameters.

Results: Similar to the grade 2 or higher obesity group, the grade 1 obesity group had a male predominance (85%) and showed a high incidence of hypertension (80%). Urinary protein excretion and renal outcome parameters were comparable between the groups. Patients with grade 1 obesity showed typical histologic features of obesity-related glomerulopathy: low glomerular density with glomerulomegaly. The glomerular density and mean glomerular volume in the grade 1 group, the grade 2 or higher group, and the kidney transplant donors with grade 1 obesity were 1.6 $\pm$ 0.8 versus 1.4 $\pm$ 0.6 versus 3.0 $\pm$ 1.1 (per mm^2) and 6.1 $\pm$ 2.1 versus 6.4 $\pm$ 1.6 versus 2.9 $\pm$ 0.8 ($\times 10^6$ μm^3), respectively.

Discussion: A glomerulopathy similar to obesity-related glomerulopathy can occur in moderately obese individuals. Renal factor(s), such as low glomerular density, may thus underlie susceptibility to this disease entity as well as BMI.

O besity-related glomerulopathy (ORG) is an established disease entity that occurs in obese individuals. Kambham *et al.*[1] reported an increased incidence of ORG among patients subjected to kidney biopsy in the United States. In that study, the number of ORG cases increased from 0.2% in 1986–1990 to 2.0% in 1996–2000. Likewise, reports from Spain, China, and Japan have suggested that the incidence of ORG is increasing, likely due to the increasing rates of obesity in the general population.[2–4]

ORG is defined as a proteinuric renal disease in patients with a body mass index (BMI) of $\geq$30 kg/m^2 and without clinical and histopathologic evidence of other renal diseases.[1,2,4] The typical clinical features of ORG include moderate or massive amounts of proteinuria without a decrease in serum levels of albumin.[1–4] Histopathologically, glomerulomegaly, and focal segmental glomerulosclerosis (FSGS) are characteristic findings,[1,2,4] likely reflecting intraglomerular hyperfiltration in these patients.[5–7] Moreover, the glomerular density (glomerular number per renal cortical area of a needle biopsy) is extremely low in patients with ORG.[8] Such an uneven glomerular density distribution in patients with ORG is unlike that in biopsies of kidney transplant donors and patients with other primary glomerular diseases. In addition, an autopsy analysis of the glomerular density in nondiseased kidneys suggested that such a low glomerular density is present only in patients with ORG, and is rare in individuals with a BMI $\geq$30 kg/m^2 in the absence of renal disease. Despite the marked glomerulomegaly in patients with ORG, the glomerular volume in kidneys of obese individuals without renal disease is only slightly greater. These results suggest that a low glomerular density associated with glomerulomegaly is a characteristic

renal histologic finding in patients with ORG, and that this difference in histopathologic measures is not simply related to BMI.

In a survey of the Japanese population, moderate obesity ($25 \leq$ BMI < 30 kg/m^2) was closely related to an increased incidence of various obesity-related diseases, and so this BMI category was defined as grade 1 obesity.[9] Recent epidemiologic studies suggest that a substantial number of patients have a disease state similar to ORG, including those with grade 1 obesity.[10–12] Currently, however, little is known regarding the renal complication(s) that can occur in patients in this BMI category. In the current study, we explored the clinicopathologic characteristics of a proteinuric renal disease in moderately obese subjects in whom renal biopsy was performed.

METHODS

Patients

This study included patients who underwent renal biopsies of native kidneys at Jikei Hospital, Tokyo, from 1999 to 2008. The exclusion criteria are shown in Table 1. Patients with renal manifestations, including renal impairment or proteinuria, before becoming obese and those who showed acute-onset nephrotic syndrome were excluded. Impaired glucose tolerance alone, as defined as an HbA1c $\geq 6.5\%$ at the time of biopsy, was not a criterion for exclusion. However, patients with a history of impaired glucose tolerance for >5 years or those with extrarenal diabetic complications, including retinopathy and neuropathy, were excluded from this study. Glomerular injuries in patients with obesity were defined morphologically as obesity-associated glomerulomegaly with or without FSGS lesions, as reported previously.[1–4,8] Patients whose renal biopsies showed clinical and histologic evidence of other primary or secondary renal diseases, including diabetic nephropathy, were excluded. Increased glomerular basement membrane thickness alone was not a criterion for exclusion, because obese patients can exhibit an increased glomerular basement membrane thickness in the absence of diabetes.[13,14] Likewise, the presence of

Table 1. The exclusion criteria

Patients who were excluded from the study
(1) Renal impairment or proteinuria before becoming obese
(2) Sudden onset of nephrotic syndrome
(3) Glucose intolerance for more than 5 yr
(4) Extrarenal manifestations of diabetes
(5) Any other glomerular disease including diabetic nephropathy
(6) Moderate-to-severe hypertensive nephrosclerosis
(7) Less than 2-yr follow-up
Not excluded
(1) Hypertension without moderate-to-severe hypertensive nephrosclerosis
(2) Thickened GBMs alone

GBM, glomerular basement membrane.

hypertension was not an exclusion criterion. The biopsy specimens of some obese hypertensive patients had moderate-to-severe vascular lesions, which were accompanied by collapsed glomeruli. Patients with such histologic features were diagnosed with hypertensive nephrosclerosis instead of glomerulopathy associated with obesity, and were excluded from this study. Patients with at least 2 years of follow-up data were included in this study.

Definitions

According to the criteria proposed by the Japan Society for the Study of Obesity, grade 1 obesity was defined as $25 \leq$ BMI < 30 kg/m^2, grade 2 as $30 \leq$ BMI < 35 kg/m^2, grade 3 as $35 \leq$ BMI < 40 kg/m^2, and grade 4 as a BMI ≥ 40 kg/m^2.[15] The biopsies of 20 patients with grade 1 obesity (group 1) and 20 patients with grade 2 or higher obesity (group 2; 16 with grade 2 and 4 with grade 3) were recruited from the renal biopsy archive during the study period. Renal biopsies of kidney transplant donors without apparent chronic kidney diseases were used for comparisons of histologic findings. Of these kidney transplant donors, 21 cases had a BMI of < 25 kg/m^2 and 10 had a grade 1 obesity. The estimated glomerular filtration rate (eGFR) was calculated using a modified 3-variable equation for the GFR of Japanese individuals:[16] eGFR $= 194 \times$ age$^{-0.287} \times$ sCr$^{-1.094}$ ($\times 0.739$ if female), where sCr is the serum level of creatinine. Hypertension was defined as a systolic blood pressure > 140 mm Hg and/or diastolic blood pressure > 90 mm Hg, or use of antihypertensive medications. Subjects using antihypertensive medications, such as angiotensin blockers, for the purpose of renoprotection despite a normal blood pressure were considered to be normotensive.

Pathologic Analysis

Renal specimens were obtained by percutaneous needle biopsy. Renal biopsies of kidney transplant donors were performed under direct vision using a needle biopsy gun. Renal biopsy specimens obtained 1 hour after renal transplantation were used for the analyses. An 18-gauge biopsy needle was used in all cases. The tissues were embedded in paraffin, cut into 3- to 4-μm sections and stained with hematoxylin and eosin, periodic acid-Schiff, Masson's trichrome, and periodic acid methenamine silver. All biopsy specimens were subjected to immunohistochemical and electron microscopy analyses. The area of interstitial fibrosis and/or tubular atrophy was evaluated semiquantitatively according to the proportion of cortical area involvement. The grade of arterial lesions was scored according to the most severe lesion within the specimen, and intimal thickening was scored by comparing the thickness of the intima

Table 2. Comparison of clinical characteristics at the time of biopsy

Variables	Grade 1 obesity, n = 20	Grade 2 or higher obesity, n = 20	P value, grade 1 versus grade 2 or higher
Age	52 ± 11	45 ± 14	0.073
Gender (%male)	85	80	0.677
BMI (kg/m^2)	26.9 ± 1.3	32.0 ± 2.4	–
Hypertension (%)	80	70	0.465
Serum creatinine (mg/dl)	1.1 ± 0.5	1.2 ± 0.7	0.812
eGFR (ml/min per 1.73 m^2)	59 ± 20	64 ± 31	0.545
Urinary protein excretion (g/d)	2.1 ± 1.5	2.0 ± 1.8	0.800
Serum albumin (g/dl)	4.3 ± 0.3	4.3 ± 0.3	0.924
Serum uric acid (mg/dl)	7.3 ± 1.6	7.5 ± 1.8	0.698
Serum total cholesterol (mg/dl)	237 ± 35	216 ± 37	0.073
Serum triglyceride (mg/dl)	282 ± 205	223 ± 122	0.273
Hemoglobin A1c (%)	5.9 ± 0.5	5.9 ± 0.5	0.676

BMI; body mass index, eGFR; estimated glomerular filtration rate.

with that of the media in the same vessel segment. We defined the severity of arterial lesions lacking intimal thickening as grade 0, those with ≤50% intimal thickening as grade 1, and those with >50% as grade 2. The grade of arteriolar lesions was evaluated based on the proportion of arterioles exhibiting hyalinosis as follows: grade 0, <5% lesions; grade 1, 5% to 25% lesions; and grade 2, >25% lesions. The percentage of glomeruli affected by segmental or global sclerosis was assessed. The variants of FSGS were determined for each glomerulus according to a classification previously reported, including tip variant, collapsing variant, cellular variant, perihilar variant, and not otherwise specified.[17] The glomerular density was determined by calculating the number of glomeruli per total renal cortical area, which was measured using a computerized image analyzer (Leica IM500, Leica Microsystems, Wetzlar, Germany). The measurement of glomerular density is strongly influenced by the degree of global sclerosis. Therefore, 2 definitions of glomerular density were applied: the number of glomeruli that were not globally sclerotic per total renal cortical area and the number of glomeruli (including globally sclerotic glomeruli) per total renal cortical area. The glomerular area was defined as the area described by the outer capillary loops of the tuft using a computerized image analyzer (Leica IM500, Leica Microsystems). The mean glomerular area (GA) was calculated by averaging the areas of all glomeruli. The mean glomerular volume (GV) was calculated from the measured GA: $GV = (GA)^{3/2} \times \beta/d$, where β is a dimensionless shape coefficient (β = 1.38 for spheres) and d is a size distribution coefficient used to adjust for variation in glomerular size.[18] The analysis used $d = 1.01$, as in previous reports.[19]

Statistical Analysis

Continuous variables are expressed as means ± SD. The variables were assessed for normality both visually (normal probability plot) and by inferential statistics (Shapiro-Wilk W and Kolmogorov-Smirnov tests). Continuous variables were compared by the t-test or the Wilcoxon rank-sum test, as appropriate. Categorical variables were expressed as percentages and compared by the χ^2 test. Values of $P < 0.05$ were considered to indicate statistical significance. All statistical analyses were performed using the SPSS software package (SPSS, Chicago, IL).

RESULTS

Clinical Characteristics at the Time of Biopsy

The clinical characteristics at the time of biopsy of patients with glomerulopathy associated with grade 1 and grade 2 or higher obesity are shown in Table 2. Compared with group 2, those in group 1 tended to be older. There was a male predominance in both groups, with 80% in group 1 having hypertension. The serum levels of creatinines and the mean eGFR values were comparable between the groups. The mean serum levels of albumin were comparable between the groups, and no patients in either group had low concentrations. The mean serum concentrations of uric acid, total cholesterol, triglyceride, and HbA1c were comparable between the groups.

Renal Outcomes

Renal outcome parameters were compared between the patients with grade 1 and those with grade 2 or higher obesity (Table 3). The average follow-up duration was 6.2 years in both groups. During the follow-up period, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers were used by 18 (90%) of the patients in group 1 and 17 (85%) in group 2. No patients in either group received corticosteroids or immunosuppressive reagents. The slopes of renal function decline during the observation period (ΔeGFR), and the frequencies of patients with a 30% decrease in eGFR at the last observation, a 50% decrease in eGFR at the last observation, and those who developed end-stage renal disease were not significantly different between the groups.

Histopathologic Findings

The histopathologic findings are shown in Table 4. Patients in group 1 had mild-to-moderate levels of chronic renal injuries, including interstitial fibrosis and/or tubular atrophy, global glomerulosclerosis, and FSGS. Among the renal biopsy specimens from these patients, 21 glomeruli from 10 patients showed FSGS lesions, 6 (29%) of which were perihilar variants and the other 15 (71%) were of the not-otherwise-specified variant. These features were similar to those in group 2. Typical FSGS lesions showing cellular, collapsing, or

Table 3. Comparison of renal outcome[a]

Variables	Grade 1 obesity, n = 20	Grade 2 or higher obesity, n = 20	P value
Duration of follow-up (yr)	6.2 ± 3.0	6.2 ± 2.8	0.944
Use of ACE-I/ARB (%)	90	85	0.633
Use of corticosteroids or immunosuppressants (%)	0	0	–
ΔeGFR (%/yr)	7.4 ± 9.2	5.8 ± 7.1	0.450
Patients with a 30% decrease in eGFR at the last observation (%)	50	40	0.525
Patients with a 50% decrease in eGFR at the last observation or those reached ESRD (%)	25	15	0.212

ACE-I, angiotensin-converting enzyme inhibitor; ARB; angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease.
[a]Patients with >2-yr follow-up were included in the analyses.

tip variants were not identified in either group. Regarding vascular lesions, the mean grades of arterial lesions and arteriole lesions were comparable.

The mean glomerular density and volume values were compared between the groups. The glomerular densities in both groups were considerably lower than that of kidney transplant donors with or without obesity. The low glomerular density in these obese patients remained evident when globally sclerotic glomeruli were included in the calculation of glomerular density. In contrast, the glomerular volume of these obese patients was markedly larger than that of the kidney transplant donors with or without obesity. The glomerular density and volume did not differ significantly between the 2 obesity groups. In addition, they did not differ significantly between the kidney transplant donors with and without obesity.

Figure 1 shows the representative renal biopsy findings in a case of kidney transplant donor (a) and a

patient with glomerulopathy associated with grade 1 obesity (b). They are observed to be quite different with regard to the density and the size of the glomeruli.

DISCUSSION

We explored the clinicopathologic characteristics of a proteinuric glomerulopathy found in individuals with grade 1 obesity ($25 \leq \text{BMI} < 30 \text{ kg/m}^2$). Our results showed that the clinical characteristics of proteinuric glomerulopathy in such patients were similar to those in patients with grade 2 or higher obesity. In addition, during an average of 6.2 years of follow-up, the renal outcome parameters, including the slope of renal function decline and the incidence of progression, did not differ significantly between the groups. Furthermore, the 2 groups exhibited similar histopathologic findings. Notably, group 1 (grade 1 obesity) had typical histologic characteristics of patients with ORG with a BMI value of $\geq 30 \text{ kg/m}^2$, that is, low glomerular density with glomerulomegaly.

The glomerular density was extremely low in proteinuric patients in both groups. Interestingly, such an uneven distribution of glomerular density in these obese patients was in sharp contrast to the widely distributed glomerular density in biopsies of kidney transplant donors with or without obesity, suggesting that the difference was not simply related to BMI. In addition, the low glomerular density in these obese patients is not likely to be due to glomerular injury, as indicated by the difference in glomerular density between the obese patients and kidney transplant donors, even when globally sclerotic glomeruli were included in the calculation of glomerular density. In addition, the glomerular density in these obese patients was

Table 4. Comparison of histopathologic findings

Variables	Grade 1 obesity, n = 20	Grade 2 or higher obesity, n = 20	P value[a]	Kidney donor (BMI < 25), n = 21	Kidney donor grade 1 obesity, n = 10	P value[b]
Interstitial fibrosis and/or tubular atrophy (%)	28 ± 21	27 ± 20	0.921	5 ± 4	9 ± 8	0.003
Glomeruli affected by global glomerulosclerosis (%)	22 ± 17	25 ± 20	0.715	5 ± 7	4 ± 6	<0.001
Patients with FSGS (%)	50	45	0.752	0	0	<0.001
Glomeruli affected by FSGS (%)	6.2 ± 8.1	5.2 ± 7.6	0.704	0	0	0.003
FSGS variant						
Glomeruli with perihilar variant (%)	1.5 ± 2.8	0.6 ± 1.5	0.198	0	0	–
Glomeruli with NOS variant (%)	4.5 ± 6.3	4.6 ± 6.9	0.968	0	0	–
Glomeruli with cellular variant (%)	0	0	–	0	0	–
Glomeruli with collapsing variant (%)	0	0	–	0	0	–
Glomeruli with tip variant (%)	0	0	–	0	0	–
Arterial lesions (grade)	0.8 ± 0.7	0.8 ± 0.8	1.000	NA	NA	–
Arteriole lesions (grade)	0.7 ± 0.7	0.6 ± 0.7	0.657	NA	NA	–
Nonsclerosed glomerular number/total cortical area (per mm^2)	1.6 ± 0.8	1.4 ± 0.6	0.373	3.3 ± 1.1	3.0 ± 1.1	0.003
Total glomerular number/total cortical area (per mm^2)	2.3 ± 1.4	1.9 ± 0.7	0.221	3.4 ± 1.2	3.1 ± 1.1	0.117
Mean glomerular volume ($\times 10^6 \mu m^3$)	6.1 ± 2.1	6.4 ± 1.6	0.582	2.4 ± 0.8	2.9 ± 0.8	<0.001

FSGS, focal segmental glomerulosclerosis; NA, not assessed; NOS, not otherwise specified.
[a]Grade 1 obesity versus grade 2 or higher obesity.
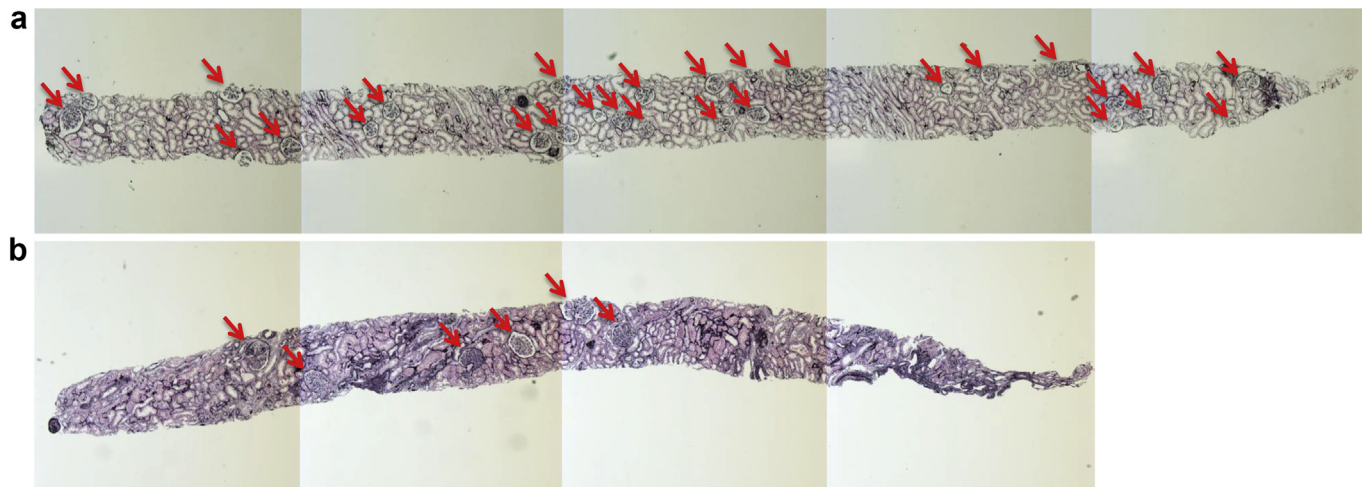[b]Grade 1 obesity versus kidney donor with grade 1 obesity.

Figure 1. Representative renal biopsy findings in light microscopy. The representative renal biopsy findings in (a) a case of a kidney transplant donor (36-year-old normotensive man with the estimated glomerular filtration rate [eGFR] of 109 ml/min per 1.73 m^2 and the body mass index [BMI] of 24.8 kg/m^2) and (b) a patient with grade 1 obesity (42-year-old hypertensive woman with the eGFR of 81 ml/min per 1.73 m^2 and the BMI of 25.5 kg/m^2) are shown. The arrows indicate nonsclerotic glomeruli. Periodic acid methenamine silver stain (original magnification ×50).

significantly lower than that in patients with IgA nephropathy or membranous nephropathy who had chronic renal injuries of similar severity.[20,21] Finally, hypertension may be associated with the low glomerular density in obesity-associated glomerulopathy, as a previous autopsy study showed a relationship between essential hypertension and kidneys with a small number of nephrons.[22] In fact, 80% of our patients in group 1 already had hypertension at the time of biopsy. However, the glomerular density did not differ between normotensive grade 1 obesity patients and hypertensive grade 1 obesity patients in the current series (data not shown), suggesting that hypertension is unlikely to be the only factor associated with the low glomerular density in these patients.

Although the origin of glomerulomegaly in patients with ORG is incompletely understood, several plausible explanations for this typical morphologic feature of ORG have been suggested. These include increased sympathetic nerve activity and/or systemic blood pressure, intraglomerular hypertension mediated by an increase in salt reabsorption and vasodilation of the afferent arterioles, and an increase in the number of glomerular capillaries due to angiogenesis-promoting factors.[7,23–25] In addition to these obesity-associated hemodynamic and humoral changes, a reduced total number of nephrons may lead to glomerular enlargement in patients with ORG. Recent autopsy studies have demonstrated a considerably greater variability in the nephron number in the general population than had been suspected.[26–28] In addition, the number of nephrons is correlated with birth weight, and low birth weight, which is associated with an inappropriate intrauterine environment, is postulated to be a risk factor for hypertension and progression of renal diseases in

later life.[29,30] Therefore, glomerular hyperfiltration may be markedly greater in individuals born with a substantially reduced number of nephrons and who then develop obesity. Together with the current results, these findings support the possibility that, in addition to obesity, renal factor(s) such as a reduced number of total nephrons may underlie the susceptibility to renal injuries associated with obesity.[31] This notion is fully consistent with the fact that the absolute risk of progressive renal deterioration in an obese individual is low and only a minority of obese individuals actually develop glomerular injury. However, whether the glomerular density in a renal biopsy specimen represents the total number of nephrons in the kidney is unclear, because data on the birth weight and total cortical volume of the kidney were not available. Therefore, the finding of a low glomerular density does not demonstrate conclusively that the subjects had a small number of glomeruli. Determining the origin of the low glomerular density requires further investigation.

A significant proportion of the FSGS lesions in our patients in each group were of the perihilar variant. Other FSGS lesions found in these patients were of the not-otherwise-specified variant; the other FSGS variants (the cellular, collapsing, and tip variants) were not identified in this study. These results are consistent with previous reports that FSGS lesions in patients with ORG are mainly of the perihilar or not-otherwise-specified variant, and other variants are identified only rarely.[1] However, the possibility remains that primary FSGS occurred in the presence of obesity in our patients, as the cause of idiopathic FSGS is unclear and idiopathic FSGS is diagnosed by the exclusion of FSGS of secondary causes.[17]

In conclusion, our findings suggest that the glomerulopathy in individuals with grade 1 obesity without other known renal diseases is similar to that in individuals with grade 2 or higher obesity in terms of the clinicopathologic features. Therefore, a glomerulopathy similar to ORG can occur in moderately obese subjects. Thus, renal factor(s) other than BMI may underlie the susceptibility to this disease entity.

DISCLOSURE

All the authors declared no competing interests.

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