Arteriolar Hyalinosis Predicts Increase in Albuminuria and GFR Decline in Normo- and Microalbuminuric Japanese Patients With Type 2 Diabetes

Tatsumi Moriya,[1] Kazuki Omura,[2] Madoka Matsubara,[1] Yuki Yoshida,[1] Kei Hayama,[1] and Motoshi Ouchi[3]

Diabetes Care 2017;40:1373–1378 | https://doi.org/10.2337/dc17-0209

OBJECTIVE

This study investigated the association between renal histology, as assessed by morphometric analysis using light (LM) and electron (EM) microscopy, and changes in urinary albumin excretion (UAE) and glomerular filtration rate (GFR) in Japanese people with type 2 diabetes in the early stages of diabetic nephropathy.

RESEARCH DESIGN AND METHODS

We performed percutaneous renal biopsies in 29 patients with type 2 diabetes (22 men, mean ± SD age 49 ± 10 years and GFR 119 ± 27 mL/min/1.73 m^2, with 15 normoalbuminuric [UAE <20 μg/min] and 14 microalbuminuric [UAE 20–200 μg/min]) to clarify which histological factors were associated with changes in UAE and GFR during 8.0 ± 3.5 years' follow-up. Glomerular structural changes including mesangial volume fraction [Vv(Mes/glom)] were estimated using EM, whereas the index of arteriolar hyalinosis (IAH) score was assessed by LM. Patients underwent annual measurement of GFR using iohexol injection with simultaneous urine collections for UAE.

RESULTS

Vv(Mes/glom) was negatively correlated with baseline and follow-up GFR but not with UAE. The IAH score was positively correlated with UAE and negatively correlated with GFR at follow-up, but it was not correlated with either UAE or GFR at baseline. GFR at follow-up was significantly decreased from baseline in patients with IAH scores ≥2.0 and significantly lower than in patients with IAH scores <2.0. Patients with IAH scores <2.0 showed no significant change in GFR during follow-up.

CONCLUSIONS

Arteriolar hyalinosis is an additional histological predictor for albuminuria increase and GFR decline in normo- and microalbuminuric Japanese people with type 2 diabetes.

It is difficult to identify patients with type 2 diabetes who are at risk for developing progressive diabetic nephropathy (DN) because of a lack of sensitivity in the commonly used measurements of glomerular filtration rate (GFR) and urinary albumin excretion (UAE). This has led to the exploration of renal histological changes as a measure of susceptibility to DN.

[1]Health Care Center, Kitasato University, Sagamihara, Kanagawa, Japan
[2]Omura Clinic, Shizuoka, Shizuoka, Japan
[3]Department of Pharmacology and Toxicology, Dokkyo Medical University School of Medicine, Mibu, Tochigi, Japan

Corresponding author: Tatsumi Moriya, moriy@kitasato-u.ac.jp.

Received 27 January 2017 and accepted 8 July 2017.

Previous studies have shown that UAE increases in parallel with mesangial expansion in patients with type 1 diabetes (1,2). Caramori et al. (3) showed that the severity of glomerular lesions, such as glomerular basement membrane (GBM) thickening and mesangial expansion, increased significantly in patients with type 1 diabetes who had normo-, micro-, or macroalbuminuria. However, there was considerable overlap in these structural parameters between these categories of UAE. In a more recent report, Caramori et al. (4) showed that GBM thickening was a risk factor for the later development of macroalbuminuria and end-stage kidney disease in patients with type 1 diabetes with normoalbuminuria and normal GFR at baseline. In Japanese patients with type 2 diabetes, GBM thickening and mesangial expansion also predicted an increase in UAE after 6 years' follow-up (5). However, the severity of the lesions also showed considerable overlap between normo- and microalbuminuria in a cross-sectional study (6). Nosadini et al. (7) showed that GBM thickening and mesangial expansion predicted GFR decline in 108 patients with type 2 diabetes who underwent research renal biopsies. They reported more progressors for GFR decline in the group with greater GBM thickening and mesangial expansion. However, they included patients with both micro- and macroalbuminuria, and consequently, the histological lesions that might predict later development of DN in type 2 diabetes remain imprecise.

Arteriolar hyalinosis is not a specific histological finding in DN. However, it is a common finding and its severity can easily be estimated using light microscopy (LM). Moreover, the presence of both afferent and efferent glomerular arteriolar hyalinosis is virtually diagnostic of DN. In our previous study, arteriolar hyalinosis was increased in patients with type 2 diabetes who were microalbuminuric compared with those with normoalbuminuria (8). In type 1 diabetes, a cross-sectional study showed that arteriolar hyalinosis was related to global glomerular sclerosis and to both creatinine clearance and UAE (9). However, to our knowledge, there are no studies in the literature on the predictive value of arteriolar hyalinosis on the changes in GFR and UAE in type 2 diabetes. Although two recent reports investigated the relationship between renal structural parameters and renal functional loss in Pima Indians (10) and Japanese patients (11), the first report did not examine arteriolar hyalinosis (10), and the second report focused almost exclusively on macroalbuminuric patients (11). Another study in Japanese patients with type 2 diabetes showed that arteriolar hyalinosis was associated with a low estimated GFR but, again, mainly focused on macroalbuminuric patients (12). Renal survival was not related to arteriolar hyalinosis in Chinese patients with type 2 diabetes also mainly with macroalbuminuria (13).

Therefore, we undertook the current study in Japanese patients with type 2 diabetes in order to identify baseline histological features seen on renal biopsy, which might identify patients susceptible to progressive DN.

RESEARCH DESIGN AND METHODS

Patients With Type 2 Diabetes

Normotensive patients with type 2 diabetes without macroalbuminuria, hematuria, or a serum creatinine ≥2.0 mg/dL (176.8 μmol/L) and without any clinical evidence of atherosclerotic disease were recruited from the outpatient clinic of Kitasato University Hospital. Patients were excluded if they were receiving antihypertensive drugs; if they had a past history of any malignant, cerebrovascular, or cardiovascular disease; and if they had recurrent infections. All of the 29 patients who were recruited gave informed, signed consent for research renal biopsy at Kitasato University Hospital (Table 1). The biopsies showed no evidence of nondiabetic renal glomerular or tubular/interstitial changes. Some of the patient data have been included in previous studies (6,8,14), but we excluded patients whose biopsies were performed for clinical indications. There were 7 women and 22 men (mean ± SD age 49 ± 10 years) with a known diabetes duration of mean ± SD 12 ± 7 years. Normoalbuminuria was defined as a UAE of <20 μg/min, whereas microalbuminuria was defined as a UAE of between 20 and 200 μg/min using 4-h urine samples collected after an iohexol injection for simultaneous estimation of GFR. Clinical examinations were performed along with a renal biopsy, and patients were followed up annually for a mean ± SD of 8.0 ± 3.5 years. The protocol for this study was approved by the Research Ethics Committee of Kitasato University School of Medicine.

Laboratory and Clinical Measurements

HbA_{1c} was measured by high-performance liquid chromatography. The value for HbA_{1c} (%) was estimated as the National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) and calculated using the following formula: HbA_{1c} (%) = HbA_{1c} (the Japan Diabetes Society) (%) + 0.4% (15). The GFR was estimated from the plasma clearance of unlabeled iohexol (14,16). Urinary albumin was measured using a turbidimetric immunoassay from 4-h urine collections taken at the time of the GFR measurement and reported as UAE. Blood pressure (BP) was measured during the initial hospitalization and at subsequent outpatient visits. Hypertension was defined as systolic BP (SBP) ≥130 mmHg, diastolic BP ≥85 mmHg, and/or receiving treatment with antihypertensive drugs. During the follow-up period, patients developing hypertension were prescribed antihypertensive therapy

Table 1—Clinical characteristics of the patients

Characteristic	
Sex, male/female	22/7
Age, years	49 ± 10
Known diabetes duration, years	12 ± 7
BMI, kg/m^2	23.1 ± 3.9
HbA_{1c}, % (mmol/mol)	8.2 ± 1.9 (66.7 ± 21.1)
UAE, μg/min	13.5 (0.0–180.2)
Normo-/microalbuminuria	15/14
GFR, mL/min/1.73 m^2	119 ± 27
SBP, mmHg	120 ± 16
DBP, mmHg	73 ± 11
DR, N/B/P	11/13/5

Data are number, mean ± SD, or median (range). DBP, diastolic blood pressure; DR, diabetic retinopathy; N/B/P, none/background/preproliferative-proliferative.

to achieve optimal BP control (<130/80 mmHg).

Morphometric Analysis of Renal Biopsies

For LM morphometric analysis, renal biopsy tissue was fixed in 10% buffered formalin and stained with periodic acid–Schiff stain. The mean glomerular volume (MGV) was determined from the LM sections at an approximate magnification of ×150 using the point counting method of Weibel and Gomez (17). Percent global glomerular sclerosis (%GS) was measured as previously described (9). At least 15 glomerular profiles (mean ± SD 24 ± 10) for each patient were measured for MGV and %GS. Interstitial volume fraction [Vv(Int/cortex)] was determined from the LM sections at an approximate magnification of ×300 by point-counting images projected onto a white surface using a projection microscope (18). The index of arteriolar hyalinosis (IAH) score was obtained by the estimation of the fraction of each arteriolar wall replaced by hyaline in one complete LM section (19). As described in the previous report (19), we calculated the IAH scores according to the following formula.

Numerator: 1 × number of arterioles with a score ≤0.25
+2 × number of arterioles with a score 0.26–0.50
+3 × number of arterioles with a score 0.50–0.75
+4 × number of arterioles with a score ≥0.76
Denominator: Total number of arterioles counted

We examined 14 ± 8 (range, 3–30) vessels per biopsy.

For electron microscopic morphometric analysis, kidney tissue was cut into cubes (~1 mm^3), fixed in 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.4), and postfixed in osmium tetroxide. These specimens were dehydrated in a graded series of ethanol and embedded in Quetol 812 (Nissin EM Co., Inc., Tokyo, Japan). All the specimens were cut into thick and 80- to 90-nm ultrathin sections and studied using a JEOL CX 100 transmission electron microscope (JEOL Ltd., Tokyo, Japan) in the Kitasato Bio-Imaging Center. Routine stereological techniques, previously described (1,6,20,21), were used to measure the GBM width, mesangial volume fraction [Vv(Mes/glom)], and the surface density of the peripheral GBM [Sv(PGBM/glom)]. Briefly, the GBM width was measured using the orthogonal intercept method (21), Vv(Mes/glom) by point counting, and Sv(PGBM/glom) by using the line-intercept method (1,6,20).

Statistical Analyses

Data are presented as mean ± SD. Relationships between renal structure and function were analyzed using regression analysis. All *P* values are two sided, with values <0.05 considered to indicate statistical significance. All statistical analyses and data management were conducted using JMP, version 10 (SAS Institute, Cary, NC).

RESULTS

Renal Function Between Baseline and Follow-up

After 8.0 ± 3.5 years of follow-up, UAE, GFR, and SBP did not differ between baseline and follow-up as a group (UAE 13.5 [range 0.0–180.2] vs. 15.9 μg/min [1.3–1,180.3], GFR 118.6 ± 27.0 vs. 105.2 ± 28.9 mL/min/1.73 m^2, and SBP 120 ± 16 vs. 127 ± 18 mmHg, respectively). There were four patients with normoalbuminuria who became microalbuminuric at follow-up, and five patients with microalbuminuria progressed to macroalbuminuria, while three patients remitted to normoalbuminuria. Seven microalbuminuric patients were prescribed renin-angiotensin-aldosterone system–blocking agents during the observation period. The levels of albuminuria did not change in seven case subjects, and six microalbuminuria case subjects did not revert to normoalbuminuria during the observation period despite being prescribed renin-angiotensin-aldosterone system blockade (data not shown). There was no significant relationship between UAE and GFR at baseline, but GFR was negatively correlated with UAE at follow-up (r = –0.41, P = 0.031).

Morphometric Analysis and Renal Function

At the Renal Biopsy

No morphometric measurements, including Vv(Mes/glom), correlated with UAE at baseline. Vv(Mes/glom) negatively correlated with baseline GFR (r = –0.388, P = 0.036). No other morphometric data, including the IAH score, correlated with GFR at baseline. The IAH score did not correlate significantly with any other morphometric measurement.

Follow-up

Vv(Mes/glom) was negatively correlated with follow-up GFR (r = –0.401, P = 0.033) (Fig. 1*A*) but did not correlate with UAE (Fig. 1*B*). On the other hand, the IAH score was negatively correlated with GFR (r = –0.491, P = 0.008) (Fig. 2*A*) and positively correlated with UAE (r = 0.420, P = 0.007) (Fig. 2*B*) at follow-up. However, none of the structural parameters, such as MGV, Vv(Int/cortex), %GS, IAH score, GBM width, Vv(Mes/glom), or Sv(PGBM/glom), showed any significant correlation with annual changes of UAE or GFR. Among the functional parameters, UAE, GFR, and SBP at baseline did not correlate significantly with UAE or GFR at follow-up, while GFR at baseline correlated with the annual change of GFR but not UAE (data not shown). The

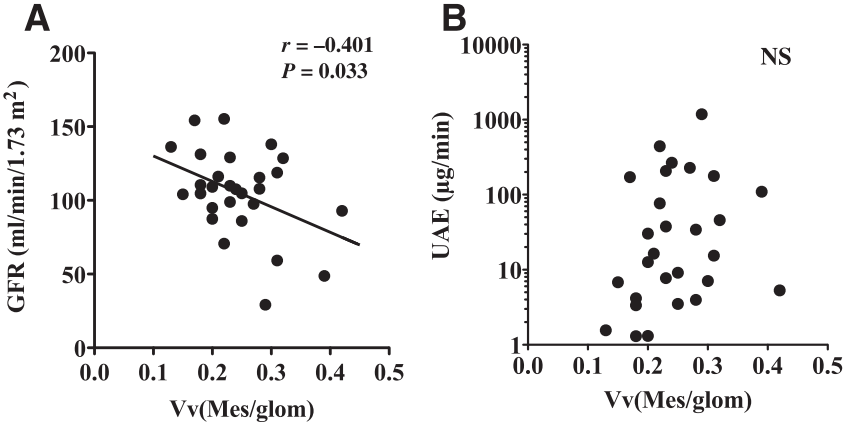

Figure 1—The relationship of Vv(Mes/glom) with GFR (*A*) and UAE (*B*) at follow-up. Vv(Mes/glom) was significantly and negatively correlated with GFR at the final observation (r = –0.401, P = 0.033). Vv(Mes/glom) did not correlate with UAE.

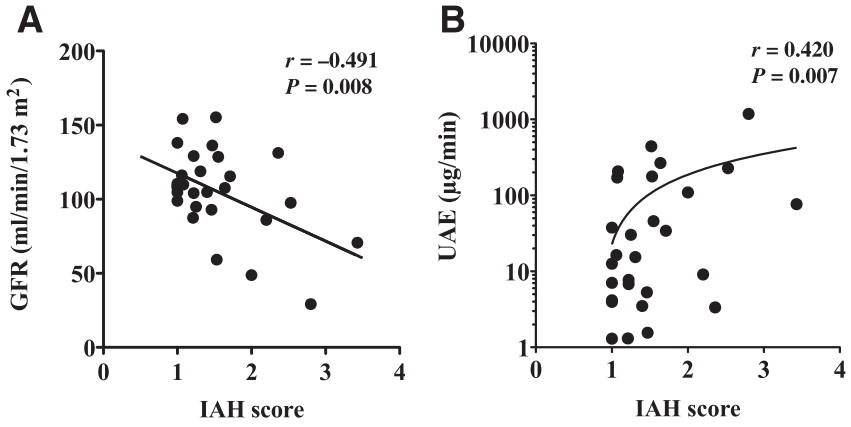

Figure 2—The relationship of the IAH score with GFR (*A*) and UAE (*B*) at follow-up. The IAH score was significantly and negatively correlated with GFR at the final observation ($r = –0.491$, $P = 0.008$). The IAH score was significantly and positively correlated with UAE at the final observation ($r = 0.420$, $P = 0.007$).

IAH score was <2.0 in 22 patients and ≥2.0 in 7 patients (Table 2). There were no significant differences in age, known duration of diabetes, HbA_{1c}, UAE at baseline, GFR at baseline, MGV, Vv(Int/cortex), %GS, GBM width, Vv(Mes/glom), or Sv(PGBM/glom) between patients with IAH scores <2.0 and ≥2.0. However, GFR at follow-up in patients with IAH scores ≥2.0 significantly decreased from baseline ($P = 0.025$) and was significantly lower than that of the patients with IAH scores <2.0 ($P = 0.005$), which did not decrease significantly from baseline (Table 2).

Stepwise Regression Analysis

We subsequently tested which histological factors, such as MGV, Vv(Int/cortex), %GS, IAH, GBM width, Vv(Mes/glom), and Sv(PGBM/glom), mostly affected follow-up GFR. Only the IAH score significantly affected GFR at the final observation ($F = 5.20$, $P = 0.036$) (Table 3). Other histological findings including mesangial expansion did not affect the final GFR.

Table 2—Clinical characteristics, renal function, and morphometric data in patients with IAH ≥2.0 or <2.0

	IAH ≥2.0	IAH <2.0	*P*
Sex, male/female	7 (5/2)	22 (17/5)	ND
Age, years	48 ± 12	49 ± 10	0.765
Known duration of diabetes, years	12 ± 8	12 ± 7	0.955
HbA_{1c}, % (mmol/mol)	8.2 ± 1.7 (66.0 ± 8.1)	8.3 ± 2.0 (66.9 ± 4.6)	0.924
UAE at baseline, μg/min	48.6 (7.3–82.4)	12.2 (0.0–180.2)	0.161
UAE at follow-up, μg/min	93.1 (3.4–1180.3)	14.0 (1.3–442.2)	0.208
P, UAE at baseline vs. follow-up	0.157	0.231	
Iohexol-GFR at baseline, mL/min/1.73 m^2	122.6 ± 28.1	117.3 ± 27.1	0.657
Iohexol-GFR at follow-up, mL/min/1.73 m^2	77.2 ± 36.2	112.9 ± 21.8	0.005
P, GFR at baseline vs. follow-up	0.025	0.443	
MGV, $\times 10^6$ μm^3	2.6 ± 0.4	3.3 ± 0.8	0.091
Vv(Int/cortex)	0.20 ± 0.05	0.21 ± 0.04	0.600
%GS	8.1 ± 13.4	3.9 ± 9.6	0.488
GBM width, nm	721 ± 127	708 ± 112	0.823
Vv(Mes/glom)	0.27 ± 0.07	0.24 ± 0.07	0.302
Sv(PGBM/glom), $\mu m^2/\mu m^3$	0.10 ± 0.02	0.11 ± 0.04	0.370
Total Mes/glom, $\times 10^6$ μm^3	0.71 ± 0.34	0.77 ± 0.26	0.638
Filtration S/G, μm^2	0.26 ± 0.04	0.37 ± 0.15	0.124

Data are *n*, median (interquartile range), or mean ± SD. Total Mes/glom, total mesangium per glomerulus; Filtration S/G, filtration surface per glomerulus; ND, not done for selection criterion.

CONCLUSIONS

In the current study, we have shown that the severity of arteriolar hyalinosis predicted both albuminuria increase and final GFR in Japanese patients with type 2 diabetes with normo- or microalbuminuria during an average 8 years of follow-up. There have been several reports of clinical or histological parameters associated with either an albuminuria increase or a GFR decline during subsequent follow-up. For example, both GBM thickening and mesangial expansion showed a significant correlation with albuminuria after 6-year follow-up in Japanese patients with type 2 diabetes (5), but this study did not report final GFR.

Arteriolar hyalinosis is not a specific histological finding in DN. However, it is often seen, and its severity can easily be estimated from LM. The presence of both afferent and efferent glomerular arteriolar hyalinosis is, however, virtually diagnostic of DN, as the other conditions where this has been reported, such as severe cyanotic heart disease and cystic fibrosis, are unlikely to be confused with diabetes. There have been few reports of the relationship between arteriolar hyalinosis and renal functional changes in DN. One study (8) showed that the IAH scores in microalbuminuric patients with type 2 diabetes were higher than those in normoalbuminuric patients, even though the IAH did not relate to chronic kidney disease stage (8). Among previous follow-up studies using renal biopsies, a Japanese study (11) did not show conclusively that albuminuria increase or renal functional decline, or both, was associated with arteriolar hyalinosis. However, the patients in that study (11) were almost all macroalbuminuric patients, even though a previous report (22) showed a significant difference in renal survival rate between the arteriolar hyalinosis scores in mainly macroalbuminuric patients. In addition, the classification of arteriolar hyalinosis severity used in that study (11) was relatively simple. Another report (12), also in Japanese patients with type 2 diabetes, showed that arteriolar hyalinosis was correlated with a low estimated GFR at the time of the renal biopsy; however, that study (12) did not show that renal outcome was related to arteriolar hyalinosis and, again, mainly focused on macroalbuminuric patients. Finally, a study that focused on

Table 3—Stepwise regression analysis (dependent variable: final GFR)

Independent variables	*F*	*P*
MGV	2.31	0.147
Vv(Int/cortex)	1.60	0.223
%GS	1.68	0.211
IAH	5.20	0.036
GBM width	0.24	0.629
Vv(Mes/glom)	2.78	0.113
Sv(PGBM/glom)	1.58	0.226

renal survival rates in Chinese patients with type 2 diabetes mainly with macroalbuminuria did not show an association between arteriolar hyalinosis and renal outcomes (13). Therefore, it remains unknown whether or not arteriolar hyalinosis is related to albuminuria increase or renal function decline, especially in the early stages of DN. Thus, our present study is, to our knowledge, the first to find that the IAH score could predict GFR loss at the early stages of DN over a relatively long follow-up period.

In the patients with type 1 diabetes who have been the subject of earlier reports, duration of diabetes was longer; GBM width and Vv(Mes/glom) were greater, and Sv(PGBM/glom) was less in patients with IAH scores $\geq$1.0 than in those with an IAH score of $<$1.0 (19). That report (19) suggested that the IAH was related to the severity of diabetic glomerulosclerosis because an IAH score of 1.0 represented almost no, or very little, arteriolar hyalinosis. However, we found no IAH relationships with other histological parameters, such as mesangial expansion or GBM thickness, in the current study of patients with type 2 diabetes. A previous cross-sectional report (8) showed that IAH in microalbuminuric patients was greater than in those with normoalbuminuria, but there was no relationship with chronic kidney disease stage. Moreover, there were no significant differences in clinical characteristics or renal function at baseline, or morphometric data, between the patients with IAH scores $<$2.0 and $\geq$2.0. We set an IAH score of 2.0 as a cutoff value because histological heterogeneity had been indicated, and some patients with type 2 diabetes exhibited striking vascular lesions, regardless of glomerulopathy (8,23). However, GFR at follow-up in patients with an IAH $\geq$2.0 was significantly decreased from baseline and lower than that seen in the patients with an IAH $<$2.0. These latter patients showed no significant change in GFR during follow-up (Table 2). In addition, stepwise regression analysis using all of the morphometric parameters indicated that only IAH significantly predicted GFR at the final observation. This finding needs verification in a larger cohort of patients and with a longer observational follow-up, but if confirmed, IAH could prove to be an independent predictor of renal functional changes in patients with type 2 diabetes.

We found no significant relationship between HbA_{1c} at the time of biopsy and IAH in the current study. Previous studies in type 1 diabetes have shown that IAH did not change 5–10 years after pancreas transplantation, although glomerular changes returned to normal or near normal after surgery (24,25). However, Fioretto et al. (26) showed that calcineurin inhibitors, which were used in that study, can induce renal arteriolar hyalinosis. Therefore, the potential histological reversibility of arteriolar hyalinosis with normoglycemia remains uncertain (25,26). Whether or not therapeutic intervention, including glycemic control, has an effect on arteriolar hyalinosis in patients with type 2 diabetes needs to be elucidated from future studies.

The strengths of the current study are the accurate assessment of arteriolar hyalinosis from light microscopic material, without the need of an electron microscopic examination, and the direct measurement of GFR with a simultaneous assessment of UAE. Moreover, to our knowledge, this is the first report that suggests that the IAH score could predict both albuminuria increase and GFR loss.

However, the current study has certain limitations. First, we analyzed only a small number of patients. Research-related renal biopsies are difficult to perform in Japan at this early stage of DN. However, we have provided evidence that one histological finding, arteriolar hyalinosis, can predict renal function loss, and this observation will hopefully lead to a larger confirmatory study with a longer follow-up.

Another limitation is that we did not perform serial biopsies, which would have provided information on the relationship between changes in IAH and other parameters and changes in GFR and UAE. A future, larger study should include a second biopsy.

In conclusion, arteriolar hyalinosis, as assessed from LM, is a potential histological predictor of albuminuria increase and GFR decline in normo- and microalbuminuric Japanese patients with type 2 diabetes. Further clinical research is warranted to elucidate the mechanism of the influence of an elevated IAH score on renal functional changes in progressive DN.

Acknowledgments. The authors thank Eriko Oda and Noriko Nemoto of the Kitasato Bio-Imaging Center for technical assistance and Robert E. Brandt, founder, Chief Executive Officer, and Chief Medical Editor of MedEd Japan, for editing and formatting the manuscript. The authors also thank Professor Rudy Bilous of Newcastle University (Malaysia) for rewriting the manuscript.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. T.M. collected and analyzed data and developed and wrote the manuscript, including reviewing the literature. K.O. analyzed data and contributed to the discussion. M.M., Y.Y., K.H., and M.O. also contributed to the discussion. T.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented in abstract form at the 75th and 77th Scientific Sessions of the American Diabetes Association, Boston, MA, 5–9 June 2015, and San Diego, CA, 9–13 June, 2017.

References

1. Fioretto P, Steffes MW, Mauer M. Glomerular structure in nonproteinuric IDDM patients with various levels of albuminuria. Diabetes 1994;43:1358–1364

2. Fioretto P, Steffes MW, Sutherland DE, Mauer M. Sequential renal biopsies in insulin-dependent diabetic patients: structural factors associated with clinical progression. Kidney Int 1995;48:1929–1935

3. Caramori ML, Kim Y, Huang C, et al. Cellular basis of diabetic nephropathy: 1. Study design and renal structural-functional relationships in patients with long-standing type 1 diabetes. Diabetes 2002;51:506–513

4. Caramori ML, Parks A, Mauer M. Renal lesions predict progression of diabetic nephropathy in type 1 diabetes. J Am Soc Nephrol 2013;24:1175–1181

5. Moriya T, Tanaka K, Hosaka T, Hirasawa Y, Fujita Y. Renal structure as an indicator for development of albuminuria in normo- and microalbuminuric type 2 diabetic patients. Diabetes Res Clin Pract 2008;82:298–304

6. Moriya T, Moriya R, Yajima Y, Steffes MW, Mauer M. Urinary albumin as an indicator of diabetic nephropathy lesions in Japanese type 2 diabetic patients. Nephron 2002;91:292–299

7. Nosadini R, Velussi M, Brocco E, et al. Course of renal function in type 2 diabetic patients with abnormalities of albumin excretion rate. Diabetes 2000;49:476–484

8. Moriya T, Suzuki Y, Inomata S, Iwano M, Kanauchi M, Haneda M. Renal histological heterogeneity and functional progress in normoalbuminuric and microalbuminuric Japanese patients with type 2 diabetes. BMJ Open Diabetes Res Care 2014;2:e000029

9. Harris RD, Steffes MW, Bilous RW, Sutherland DE, Mauer SM. Global glomerular sclerosis and glomerular arteriolar hyalinosis in insulin dependent diabetes. Kidney Int 1991;40:107–114
10. Fufaa GD, Weil EJ, Lemley KV, et al. Structural predictors of loss of renal function in American Indians with type 2 diabetes. Clin J Am Soc Nephrol 2016;11:254–261
11. Mise K, Hoshino J, Ueno T, et al. Prognostic value of tubulointerstitial lesions, urinary N-Acetyl-β-d-glucosaminidase, and urinary β2-microglobulin in patients with type 2 diabetes and biopsy-proven diabetic nephropathy. Clin J Am Soc Nephrol 2016;11:593–601
12. Shimizu M, Furuichi K, Toyama T, et al.; Kanazawa Study Group for Renal Diseases and Hypertension. Long-term outcomes of Japanese type 2 diabetic patients with biopsy-proven diabetic nephropathy. Diabetes Care 2013;36:3655–3662
13. Li L, Zhang X, Li Z, et al. Renal pathological implications in type 2 diabetes mellitus patients with renal involvement. J Diabetes Complications 2017;31:114–121
14. Moriya T, Tsuchiya A, Okizaki S, Hayashi A, Tanaka K, Shichiri M. Glomerular hyperfiltration and increased glomerular filtration surface are associated with renal function decline in normo- and microalbuminuric type 2 diabetes. Kidney Int 2012;81:486–493
15. Seino Y, Nanjo K, Tajima N, et al.; Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus. Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. J Diabetes Investig 2010;1:212–228
16. Jin Y, Moriya T, Tanaka K, Matsubara M, Fujita Y. Glomerular hyperfiltration in non-proteinuric and non-hypertensive Japanese type 2 diabetic patients. Diabetes Res Clin Pract 2006;71:264–271
17. Weibel ER. Practical methods for biological morphometry. In *Stereological Methods*. Vol. 1. New York, Academic Press, 1979
18. Lane PH, Steffes MW, Fioretto P, Mauer SM. Renal interstitial expansion in insulin-dependent diabetes mellitus. Kidney Int 1993;43:661–667
19. Drummond K, Mauer M; International Diabetic Nephropathy Study Group. The early natural history of nephropathy in type 1 diabetes: II. Early renal structural changes in type 1 diabetes. Diabetes 2002;51:1580–1587
20. Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC. Structural-functional relationships in diabetic nephropathy. J Clin Invest 1984;74:1143–1155
21. Jensen EB, Gundersen HJ, Osterby R. Determination of membrane thickness distribution from orthogonal intercepts. J Microsc 1979;115:19–33
22. Mise K, Hoshino J, Ubara Y, et al. Renal prognosis a long time after renal biopsy on patients with diabetic nephropathy. Nephrol Dial Transplant 2014;29:109–118
23. Fioretto P, Mauer M, Brocco E, et al. Patterns of renal injury in NIDDM patients with microalbuminuria. Diabetologia 1996;39:1569–1576
24. Fioretto P, Steffes MW, Sutherland DE, Goetz FC, Mauer M. Reversal of lesions of diabetic nephropathy after pancreas transplantation. N Engl J Med 1998;339:69–75
25. Fioretto P, Sutherland DE, Najafian B, Mauer M. Remodeling of renal interstitial and tubular lesions in pancreas transplant recipients. Kidney Int 2006;69:907–912
26. Fioretto P, Najafian B, Sutherland DE, Mauer M. Tacrolimus and cyclosporine nephrotoxicity in native kidneys of pancreas transplant recipients. Clin J Am Soc Nephrol 2011;6:101–106