Nephron

Clinical Practice: Research Article

Nephron
DOI: 10.1159/000535875

Received: May 27, 2023
Accepted: November 28, 2023
Published online: December 20, 2023

Arteriolar Hyalinosis Predicts the Onset of Both Macroalbuminuria and Impaired Renal Function in Patients with Type 2 Diabetes

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Keywords
Renal biopsy · Diabetic nephropathy · Macroalbuminuria · Impaired renal function · Arteriolar hyalinosis

Abstract
Introduction: Arteriolar hyalinosis (AH) has been shown to be associated with albuminuria and GFR. In this study, we investigated whether or not index of AH (IAH) is a predictor of the onset of macroalbuminuria and impaired renal function (eGFR <60 mL/min/1.73 m^2 [eGFR <60]) in type 2 diabetic patients with early diabetic nephropathy. ***Methods:*** The study population consisted of 35 patients with type 2 diabetes (25 men; age: 47 ± 9 years; eGFR: 92.7 ± 18.0 mL/min/1.73 m^2) with normo- or microalbuminuria who underwent percutaneous renal biopsy. These patients were followed for at least 5 (18 ± 6, range: 6–28) years. The study endpoint was the onset of macroalbuminuria or eGFR <60. Light and electron microscopy-based morphometric analyses were performed to quantitatively evaluate glomerular and interstitial structural changes. ***Results:*** During the observation period, 9 out of the 35 patients progressed to macroalbuminuria, and 15 out of the 35 patients developed eGFR <60. The annual rate of eGFR decline was significantly correlated with IAH ($r = -0.40$, $p = 0.016$). Kaplan-Meier analysis demonstrated that AH was associated with a significantly higher risk of onset of macroalbuminuria and eGFR <60, and microalbuminuria is associated with the onset of macroalbuminuria but not the onset of eGFR <60. ***Conclusions:*** Aggravated AH is a histological risk factor which predicts the onset of macroalbuminuria and eGFR <60 in patients with type 2 diabetes. These findings provide novel insights into the mechanism of progression of diabetic nephropathy.

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Introduction

As end-stage renal disease (ESRD) is a serious diabetic complication [1–4], it has been of interest to identify biological or renal histological markers that indicate renal function decline in diabetic nephropathy. Macroalbuminuria and estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 (eGFR <60) are thought to be independent predictors of renal functional decline leading to ESRD [5]. Therefore, many researchers have tried to identify clinical factors that lead to macroalbuminuria or eGFR <60. Glomerular basement membrane (GBM) thickening was shown to be a risk factor for the development of macroalbuminuria and ESRD in type 1 diabetes patients with normoalbuminuria [6]. In Japanese patients with type 2 diabetes, GBM thickening

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Table 1. Clinical characteristics of the patients at the time of renal biopsy

Age, years	47±9
Sex (M/F)	23/12
Known duration of diabetes, years	11±7
Body mass index, kg/m^2	23.0±4.1
HbA_{1c} (NGSP[%]) (IFCC [mmol/mol])	8.4±2.2 (68.3±24.2)
U-Alb/Cr,* mg/gCr	30.5 (9.1–68.4)
Normoalbuminuria/microalbuminuria	18/17
eGFR, mL/min/1.73 m^2	92.7±18.0
SBP, mm Hg	126±16
DBP, mm Hg	75±11

Data are shown as means ± SD, number, or median (interquartile range)*. M, male; F, female; HbA_{1c}, hemoglobinA1c; NGSP, National Glycohemoglobin Standardization Program; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; U-Alb/Cr, urinary albumin creatinine ratio; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.

and mesangial expansion were found to predict an increase in urinary albumin creatinine ratio (U-Alb/Cr) after 6.4 ± 1.8 years of follow-up (mean ± standard deviation [SD]) [7]. A research biopsy study demonstrated that GBM thickening and mesangial expansion predicted GFR decline in patients with type 2 diabetes with micro- or macroalbuminuria [8]. More recently, a study based on renal biopsies of Native American patients mainly with type 2 diabetes with normo- or microalbuminuria demonstrated that changes in glomerular structure, including mesangial expansion, predicted the loss of renal function [9]. Arteriolar hyalinosis (AH) is a histological characteristic of kidney disease, although it is not specific to diabetic nephropathy, and we showed that it predicts a lower GFR and higher U-Alb/Cr at the end of a 8.0 ± 3.5 year observation in patients with early diabetic nephropathy [10]. In addition, AH was also found to be associated with a rapid GFR decline during 11.0 ± 3.0 years of observation in patients with early diabetic nephropathy [11].

In the present study, we hypothesized that AH can predict the onset of macroalbuminuria or eGFR <60 in type 2 diabetes patients with biopsy-proven diabetic nephropathy, who have normo- or microalbuminuria in addition to other clinical or histological characteristics of diabetic nephropathy.

Material and Methods

Study Design and Population

Patients with type 2 diabetes with normo- or microalbuminuria were recruited at the outpatient clinic of Kitasato University Hospital. The following patients were excluded from this initial recruitment: patients with hypertension or who were taking antihypertensive drugs; patients with hematuria or a serum creatinine concentration of >2.0 mg/dL (176.8 mmol/L); patients with a history of any malignant, cerebrovascular, or cardiovascular disease; and patients with recurrent infections. All of the patients were fully informed about the study, and the subjects who gave their consent to participate in the present study underwent percutaneous renal research biopsies at Kitasato University Hospital (Table 1). The patients showed no evidence of nondiabetic renal glomerular or tubular/interstitial changes. Some of the patients in the present study had been included in certain parts of our previous studies [7, 10–13]. However, they were excluded if they had undergone biopsies based on clinical indications. In addition, patients with an observation period of less than 5 years after biopsy were excluded. Therefore, the final study population included 12 women and 23 men (mean age: 47 ± 9 years old) with a known diabetes duration of 11 ± 7 years. All 35 patients were annually assessed by clinical examinations, including eGFR and urinary albumin analysis, for a mean period of 18.0 ± 6.0 (range: 6–28) years. Eighteen patients had normoalbuminuria and 17 had microalbuminuria. Baseline eGFR was 92.7 ± 18.0 mL/min/1.73 m^2 and blood pressure was 126 ± 16/75 ± 11 mm Hg. The study endpoint was the onset of macroalbuminuria, eGFR <60, or March 2020.

The onset of macroalbuminuria and eGFR <60 were defined as the first date when a patient had macroalbuminuria in 2 consecutive spot urine samples and when the patient had eGFR <60 in 2 consecutive blood samples, respectively.

Normal Control Subjects

Renal biopsy reference values of normal controls were obtained from 9 living renal transplant donors (2 men and 7 women; age 51 ± 8 years) 1 h after transplantation. All 9 subjects underwent the 75 g oral glucose tolerance test, and the results showed normal glucose tolerance. The subjects were negative for dipstick proteinuria and had normal blood pressure.

Laboratory and Clinical Measurements

HbA_{1c} level was measured by high-performance liquid chromatography. The HbA_{1c} (%) value was estimated as the National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) using the following formula: HbA_{1c} (%) = HbA_{1c} (Japan Diabetes Society [JDS]) (%) + 0.4%, considering the relative expression of HbA_{1c} (JDS) (%) measured using the previous Japanese standard substance and measurement methods and HbA_{1c} (NGSP), followed by conversion to HbA_{1c} (International Federation of Clinical Chemistry). Urinary albumin was measured by a turbidimetric immunoassay using spot urine samples and shown as U-Alb/Cr. Normo- and microalbuminuria were defined as U-Alb/Cr <30 mg/gCr and 30–300 mg/gCr, respectively. Blood pressure (BP) in the sitting position was measured during the initial hospitalization and at outpatient visits by nursing staff. Hypertension was defined as a systolic BP of ≥130 mm Hg, a diastolic BP of ≥85 mm Hg, and/or the use of antihypertensive drugs. During the follow-up period, patients who developed hypertension were prescribed antihypertensive drugs for optimal BP control (<130/80 mm Hg).

Morphometric Analysis of Renal Biopsy Specimens

For morphometric analysis by light microscopy (LM), renal biopsy specimens from diabetic patients were fixed in 10% buffered formalin and subjected to periodic acid-Schiff staining. The mean glomerular volume (MGV) of the LM sections was

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evaluated at an approximate magnification of ×150 by the point counting method using the glomerular tuft area and calculated using the formula established by Weibel [14]. Percent global glomerular sclerosis (%GS) was measured as described previously [15]. At least 15 glomerular profiles (24 ± 10, mean ± SD) for each patient were measured to determine MGV and %GS values. The interstitial volume fraction (Vv [Int/cortex]) was determined using LM sections at an approximate magnification of ×300 by point-counting images projected onto a white surface with a projection microscope [16]. The index of AH (IAH) was obtained by estimating the fraction of each arteriolar wall that was replaced by hyaline in an entire LM section [10, 17]. As described in a previous report [10], IAH scores were calculated according to the following formula. An average of 15 (range: 2–55) vessels were examined per biopsy.

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

For morphometric analyses by electron microscopy (EM), kidney tissues were cut into cubes (size: approximately 1 mm^3), fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), and post-fixed in osmium tetroxide. The specimens were then dehydrated in a graded ethanol series and embedded in Quetol 812 (Nissin EM, Inc., Tokyo, Japan). All specimens were cut into thick and 80–90 nm ultrathin sections and observed under a JEOL CX 100 transmission electron microscope (JEOL, Tokyo, Japan) in the Kitasato Bio-Imaging Center. Routine stereologic techniques, which have been previously described [18–21], were used to measure GBM width, mesangial fractional volume (Vv[Mes/glom]), and the surface density of the peripheral GBM (Sv[PGBM/glom]). In brief, GBM width was measured using the orthogonal intercept method [21], Vv(Mes/glom) was measured by point counting, and Sv (PGBM/glom) was measured using the line-intercept method [18–20]. At least 2 (usually 3) glomeruli were used for morphometric analysis by EM.

Statistical Analyses

Data are presented as the mean ± SD, with the exception of U-Alb/Cr, which is presented as the median (interquartile range), because it is not normally distributed. The associations among the morphological data and the annual change rate in GFR and U-Alb/Cr were evaluated using linear and nonlinear regression analyses. The risk of developing macroalbuminuria and eGFR <60 was calculated using Kaplan-Meier analysis. p values of less than 0.05 were considered to indicate statistical significance. In addition, multivariate regression analysis was performed to investigate which serological and renal histological factors affect the onset of macroalbuminuria or eGFR <60.

Results

Morphometric Analysis of Renal Biopsy Specimens

To quantitatively evaluate glomerular and interstitial structural changes, LM- and EM-based morphometric analyses were performed. As shown in Table 2, MGV, Vv(Int/cortex), IAH, GBM, and Vv(Mes/glom) were markedly higher in patients with type 2 diabetes than in the control subjects, whereas there was no significant difference in percent glomerular sclerosis and Sv(PGBM/glom) between the 2 groups.

Renal Function between Baseline and Follow-Up

During the observation period, the eGFR decreased from 92.7 ± 18.0 mL/min/1.73 m^2 at baseline to 72.5 ± 24.5 mL/min/1.73 m^2 (p = 0.0002), and U-Alb/Cr increased from 30.5 (9.1–68.4) to 44.9 (10.1–255.2) mg/gCr (p = 0.0299) in patients with diabetes. The annual rate of increase in U-Alb/Cr was 52.2 ± 146.1 mg/gCr/year, and the annual decrease rate of eGFR was −2.1 ± 2.4 mL/min/1.73 m^2/year.

Eight of the 35 patients first developed macroalbuminuria, and 6 of these patients subsequently developed eGFR <60 during the observation period (the remaining 2 only had macroalbuminuria). On the other hand, 9 of the 35 patients first developed eGFR <60, and 1 of these patients subsequently developed macroalbuminuria during the observation period (the remaining 8 only had eGFR <60).

Risk of Developing Macroalbuminuria and eGFR <60

The risk of developing macroalbuminuria and eGFR <60 was evaluated using Kaplan-Meier analysis. As our previous reports [10, 11] showed that GFR was significantly and rapidly decreased during the 8 years of follow-up in patients with an IAH score ≥2.0, we compared the onset of macroalbuminuria and eGFR <60 between patients with an IAH score ≥2.0 and <2.0. In addition, patients were divided into 2 groups using other histological parameters, in which the patients had mean values of +2 SD of normal control subjects. Vv(Mes/glom) was used as an indicator of mesangial expansion, and patients were classified into Vv(Mes/glom) ≥ 0.25 and <0.25. The patients were also classified into 2 groups of normo- and microalbuminuria at baseline.

Figure 1 shows the results of Kaplan-Meier analysis for the duration to macroalbuminuria onset of patients divided by IAH score, mesangial volume fraction, albuminuria grade, and HbA_{1c} level. Patients with an IAH score ≥2.0 (Fig. 1a), Vv(Mes/glom) ≥ 0.25 (Fig. 1b), and microalbuminuria (Fig. 1c) had a higher risk of onset of macroalbuminuria than patients with an IAH score <2.0 (p = 0.007), Vv(Mes/glom) < 0.25 (p = 0.008), and normoalbuminuria (p = 0.006), respectively. On the other hand, HbA_{1c} ≥7.0% (Fig. 1d) was not associated with the onset of macroalbuminuria (p = 0.091).

Figure 2 shows the results of Kaplan-Meier analysis for the duration of eGFR <60 onset of patients divided by IAH score, mesangial volume fraction, albuminuria

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Table 2. Morphometric characteristics of renal biopsy samples observed by light and electron microscopy

	Patients	Normal controls	*p* value
MGV, $\times 10^6$ μm^3	3.0±0.9	1.4±0.5	<0.0001
%GS*	0 (0–1.43)	0 (0–15.4)	0.110
Vv(Int/cortex)	0.19±0.04	0.10±0.01	<0.0001
IAH score	1.6±0.7	1.0±0.0	0.003
GBM width, nm	654±148	296±43	<0.0001
Vv(Mes/glom)	0.25±0.01	0.19±0.03	0.009
Sv(PGBM/glom), μm^2/μm^3	0.11±0.03	0.12±0.02	0.319

Data are shown as means ± SD, or median (interquatile range)*. MGV, mean glomerular volume; %GS, percent global glomerular sclerosis; Vv(Int/cortex), interstitial volume fraction; IAH, index of arteriolar hyalinosis; GBM, glomerular basement membrane; Vv(Mes/glom), mesangial volume fraction; Sv(PGBM/glom), surface density of peripheral GBM.

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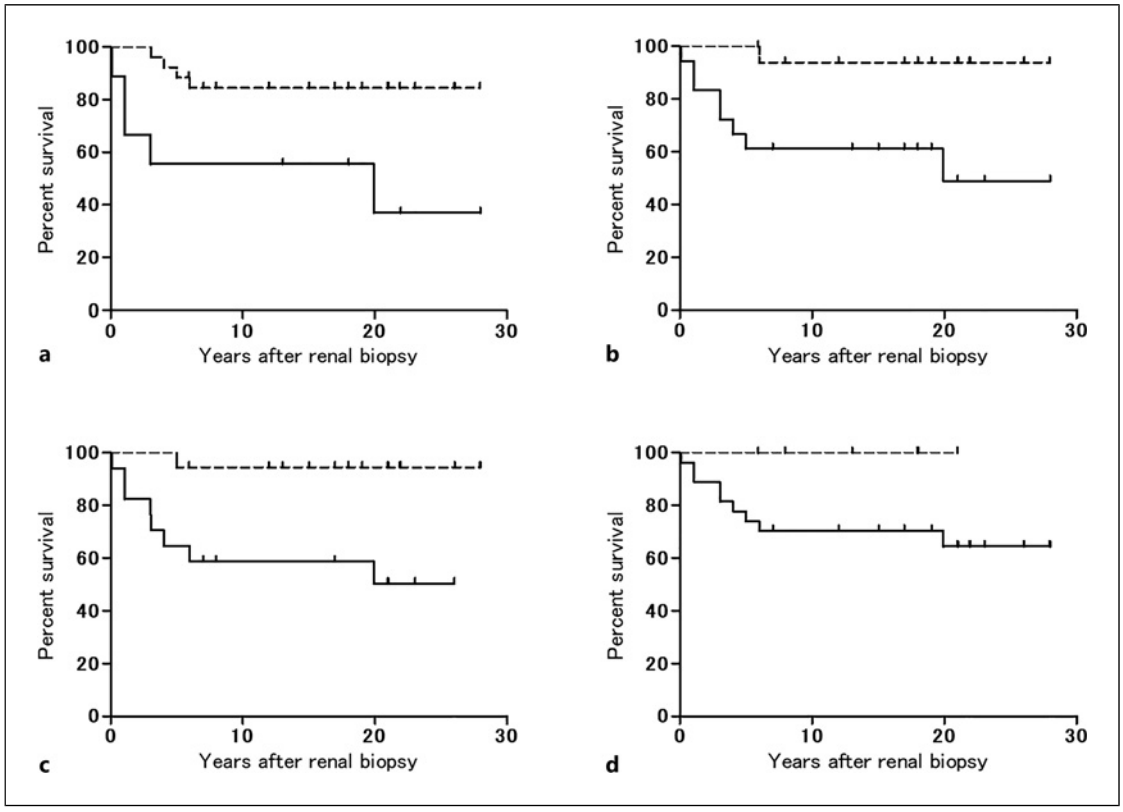

Fig. 1. Kaplan-Meier analysis of the duration to onset of macroalbuminuria in patients with different IAH scores, Vv(Mes/glom), albuminuria grades, and HbA_{1c} levels. **a** IAH score ≥2.0 (solid line) was associated with a significantly higher risk of macroalbuminuria onset than IAH score <2.0 (dashed line) (logrank $\chi^2 = 7.21$, $p = 0.007$). **b** Vv(Mes/glom) ≥ 0.25 (solid line) was associated with a significantly higher risk of macroalbuminuria onset than Vv(Mes/glom) < 0.25 (dashed line) (logrank $\chi^2 = 7.14$, $p = 0.008$). **c** Microalbuminuria (solid line) was associated with a significantly higher risk of macroalbuminuria onset than normoalbuminuria (dashed line) (logrank $\chi^2 = 7.43$, $p = 0.006$). **d** HbA_{1c} ≥7.0% (solid line) was not associated with a risk of macroalbuminuria onset, as HbA_{1c} <7.0% (dashed line) (logrank $\chi^2 = 2.86$, $p = 0.091$).

grade, and HbA_{1c} level. Patients with an IAH score ≥2.0 (Fig. 2a) and Vv(Mes/glom) ≥ 0.25 (Fig. 2b) had a higher risk of onset of eGFR <60 than patients with an IAH score <2.0 ($p = 0.012$) and Vv(Mes/glom) < 0.25 ($p = 0.021$), respectively, but the presence of microalbuminuria (Fig. 2c) and HbA_{1c} ≥7.0% (Fig. 2d) were not

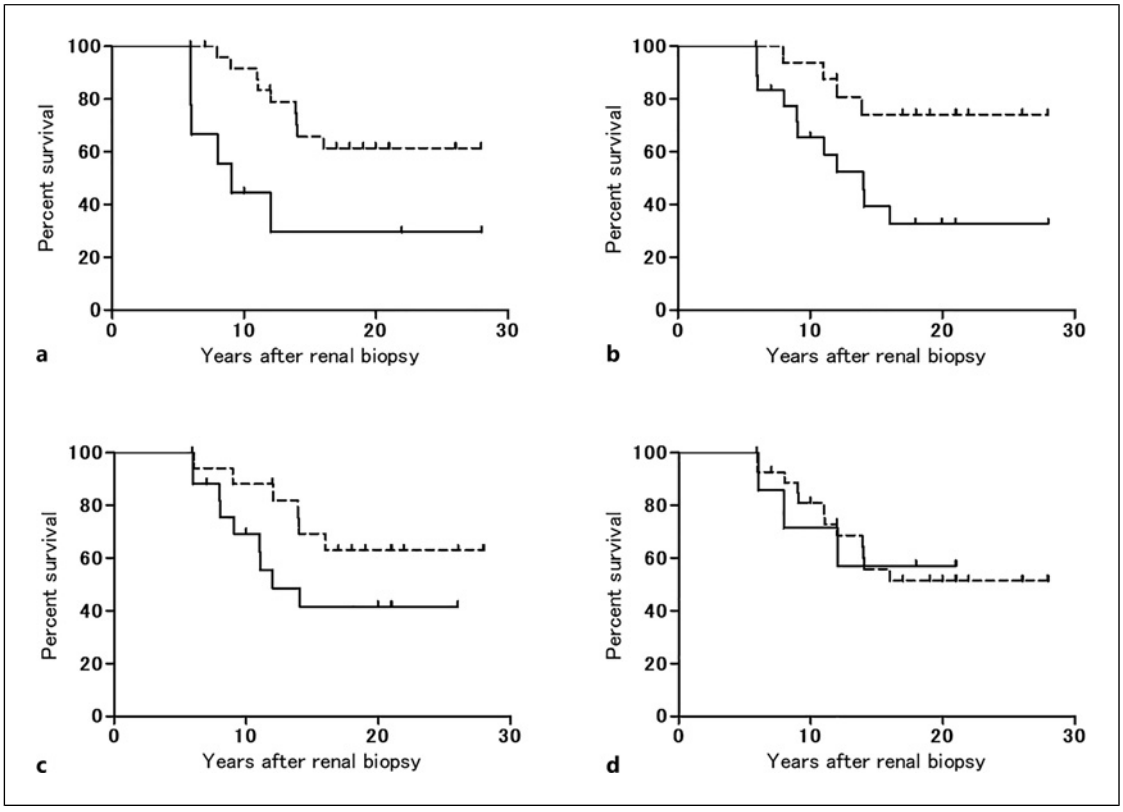

Fig. 2. Kaplan-Meier analysis of the duration to onset of eGFR <60 in patients with different IAH scores, Vv(Mes/glom), albuminuria grades, and HbA_{1c} levels. **a** IAH score ≥2.0 (solid line) was associated with a significantly higher risk of eGFR <60 onset than IAH score <2.0 (dashed line) (logrank $\chi^2 = 6.24$, $p = 0.013$). **b** Vv(Mes/glom) ≥0.25 (solid line) was associated with a significantly higher risk of eGFR <60 onset than Vv(Mes/glom) < 0.25 (dashed line) (logrank $\chi^2 = 5.37$, $p = 0.021$). **c** Microalbuminuria (solid line) was not associated with a risk of eGFR <60 onset, as normoalbuminuria (dashed line) (logrank $\chi^2 = 2.22$, $p = 0.136$). **d** HbA_{1c} ≥7.0% (solid line) was not associated with a risk of eGFR <60 onset, as HbA_{1c} <7.0% (dashed line) (logrank $\chi^2 = 0.01$, $p = 0.942$).

associated with the onset of eGFR <60 ($p = 0.136$ and $p = 0.942$, respectively.

Other structural parameters of the kidney tissue specimens were also analyzed using the Kaplan-Meier curve. As risk factors for macroalbuminuria and eGFR <60, MGV ($p = 0.083$ and $p = 0.189$, respectively), %GS ($p = 0.374$ and $p = 0.239$, respectively), Vv(Int/cortex) ($p = 0.995$ and $p = 0.401$, respectively), GBM width ($p = 0.594$ and $p = 0.433$, respectively), and Sv(PGBM/glom) ($p = 0.931$ and $p = 0.300$, respectively) did not reach statistical significance.

Serological and Histological Factors Affecting the Onset of Macroalbuminuria and eGFR <60

We next tested which serological and histological factors most strongly affected the onset of macroalbuminuria and eGFR <60. On multivariate analysis, U-Alb/Cr and IAH showed a significant association with the onset of macroalbuminuria ($p = 0.008$ and $p = 0.036$, respectively), whereas Vv(Mes/glom) and HbA_{1c} did not affect the onset of macroalbuminuria (Table 3). There were no factors significantly affecting the onset of eGFR <60, although IAH had a tendency of an association with the onset of eGFR <60 ($p = 0.060$) (Table 4).

Discussion

In the present study, we showed that AH as well as mesangial expansion at baseline can predict the onset of both macroalbuminuria and eGFR <60 in patients with type 2 diabetes with normo- and microalbuminuria with normal renal function, regardless of HbA_{1c} level and albuminuria grade. Although the association between these phenomena and AH has not been clearly demonstrated in patients with type 2 diabetes by renal biopsy,

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Table 3. Multivariate analysis of macroalbuminuria onset

Variable	Coefficient	95% CI	*p* value
HbA_{1c} (NGSP[%])	0.036	−0.42 to 0.61	0.877
U-Alb/Cr, mg/gCr	−0.025	−0.05 to −0.01	0.008
Vv(Mes/glom)	−15.025	−43.43 to 4.86	0.185
IAH score	−2.072	−4.55 to −0.38	0.036

CI, confidence interval; HbA_{1c}, hemoglobinA1c; NGSP, National Glycohemoglobin Standardization Program; U-Alb/Cr, urinary albumin creatinine ratio; Vv(Mes/glom), mesangial volume fraction; IAH, index of arteriolar hyalinosis.

Table 4. Multivariate analysis of onset of eGFR <60

Variable	Coefficient	95% CI	*p* value
HbA_{1c} (NGSP[%])	0.102	−0.28 to 0.52	0.592
U-Alb/Cr, mg/gCr	−0.034	−0.02 to 0.00	0.310
Vv(Mes/glom)	−7.214	−22.61 to 6.05	0.311
IAH score	−1.304	−2.89 to −0.06	0.060

CI, confidence interval; HbA_{1c}, hemoglobinA1c; NGSP, National Glycohemoglobin Standardization Program; U-Alb/Cr, urinary albumin creatinine ratio; Vv(Mes/glom), mesangial volume fraction; IAH, index of arteriolar hyalinosis.

our findings suggest that AH is a histological risk factor for the aggravation of diabetic nephropathy. Our present findings first demonstrated that histological changes, such as AH, have a clinical effect on the onset of macroalbuminuria and eGFR <60, rather than on the absolute value or the annual change of U-Alb/Cr and eGFR [10, 11]. In contrast, microalbuminuria was not associated with the onset of eGFR <60 in the present study. Thus, these findings demonstrated a novel link between AH and the progression of diabetic nephropathy, although less invasive alternatives to IAH should be investigated for future clinical applications. Further studies are needed to clarify the molecular mechanisms by which AH causes renal damage.

It is well known that the typical clinical course of diabetic nephropathy is an increase in urinary albumin from microalbuminuria to macroalbuminuria, which is followed by a decline in renal function, eventually to ESRD. A change from microalbuminuria to macroalbuminuria takes more than 10 years after the onset of diabetes in both patients with type 1 and 2 diabetes [22], whereas therapeutic intervention for decreasing U-Alb/Cr was reported to reduce the risk of ESRD. A cohort study of about 20,000 people, of whom 61% were patients with diabetes, reported that a 4-fold increase in U-Alb/Cr within a 2-year period was associated with a 3.08-times higher risk of ESRD, whereas a 4-fold decrease in U-Alb/Cr was associated with a 0.34-times lower risk of ESRD than patients with a stable U-Alb/Cr [23]. A recent meta-analysis also showed that a 30% reduction in U-Alb/Cr within 2 years decreased the risk of ESRD by 22% [24]. Thus, increased albuminuria has been shown to be a predictor of ESRD during a long duration of diabetes.

On the other hand, it has recently been demonstrated that the clinical course of diabetic nephropathy is diverse, with various types [25, 26]. For example, GFR can decrease before the presentation of micro- or macroalbuminuria, and urinary albumin excretion can increase without a decrease in GFR. In fact, in the present study, 8 of the 15 patients who progressed to eGFR <60 did not have macroalbuminuria. A previous study reported that eGFR itself is an important biomarker of chronic kidney disease in patients with normoalbuminuria [27]. In addition, 32% of the 68 type 2 diabetes patients with normoalbuminuria showed deterioration of renal function with an annual GFR decrease rate of 3.3% or more [28]. Furthermore, Ninomiya et al. [5] previously reported that macroalbuminuria and eGFR <60 are independent predictors of impaired renal function that eventually lead to ESRD.

Not only eGFR and/or urinary albumin excretion but also pathological findings, such as GBM and mesangial expansion, are important prognostic factors for diabetic nephropathy [29], which are involved in the development of macroalbuminuria and ESRD [30]. More importantly, it is noted that histological changes of diabetic nephropathy can be observed in patients with normoalbuminuria before abnormal laboratory data are observed [19, 20]. In an analysis of 600 patients from 13 institutions in Japan, 78.0% of patients with preserved eGFR and normoalbuminuria already showed diffuse glomerular lesions, and more than half of the patients had interstitial fibrosis and tubular atrophy, interstitial cell infiltration, AH, and intimal thickening [31]. Moreover, a high frequency of global glomerular sclerosis in patients with type 2 diabetes with normo- and microalbuminuria would participate in loss of GFR [13]. These studies indicate the importance of pathological findings in the progression of nephropathy in the normoalbuminuric phase.

AH is often observed in patients with diabetic nephropathy, and its severity can easily be estimated by LM, although this is not specific for diabetic nephropathy. A previous study of patients with type 1 diabetes [15]

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showed a positive association between IAH and %GS and demonstrated that both IAH and global sclerosis are significantly negatively associated with creatinine clearance. Another study of kidney biopsies from 94 patients with type 1 diabetes with normoalbuminuria showed that higher IAH values are associated with the progression of renal failure [6]. We previously reported that IAH predicted low GFR and high U-Alb/Cr and a rapid decrease in GFR in patients with early diabetic nephropathy [10, 11]. Our present study further demonstrated that patients with an IAH score ≥2.0 had a higher risk of developing both macroalbuminuria and eGFR <60 than other patients. Taken together, a higher IAH appears to have a substantial effect on the onset of macroalbuminuria and eGFR <60 in patients with type 2 diabetes with normo- or microalbuminuria.

It remains unclear as to why AH predicts the onset of macroalbuminuria and eGFR <60. Whereas some previous reports showed no clear association between AH and the progression of ESRD [32–34], AH was shown to be associated with the initiation of renal replacement therapy [35] and lower renal survival [36]. Salvatore et al. [37] reported a higher percentage of renal arterioles with luminal stenosis in patients with diabetic glomerulosclerosis. Furuichi et al. [38] demonstrated that arteriolar hyalinosis as well as arteriosclerosis with intimal thickening are frequently observed during the early stage of diabetic nephropathy. These previous findings indicate that AH may cause luminal narrowing of the renal arterioles, which possibly leads to glomerular ischemia, although there were no ischemic changes observed in the arteriolar lesions in our samples.

The strength of the present study is that we performed research-associated renal biopsies and an 18-year-long observation to elucidate histological factors that predict the onset of both macroalbuminuria and eGFR <60. We found that IAH was a predictor of the above phenomena as well as mesangial expansion, which is a well-known predictor of reduced renal function, whereas albuminuria was not associated with the onset of eGFR <60. In addition, IAH can easily be evaluated using LM data, whereas Vv(Mes/glom) can only be evaluated by EM data. Thus, IAH appears to be a unique and useful predictor of the progression of diabetic nephropathy, and therefore it would be ideal to evaluate IAH as early as possible. There are inevitable hurdles of renal biopsy, which has possible complications, such as bleeding and pain. In addition, cost effectiveness should be taken into account. Probably due to these reasons and others, renal biopsies for the purpose of research are rarely performed in patients at an early stage of diabetic nephropathy. Therefore, in the future, it will be crucial to identify the type of patients who will benefit from undergoing a renal biopsy at an early stage.

The present study has some limitations. First, we only analyzed a small number of patients, and we could not evaluate the effects of AH in each albuminuria category, i.e., normoalbuminuria and microalbuminuria. Second, the number of arterioles analyzed to determine IAH was relatively small, which might have affected the conclusion of the association between IAH and the functional parameters. Last, none of the study participants were receiving renoprotective therapies at baseline. However, because there were no restrictions on medications administered after the renal biopsy, we did not consider the possible effects of the renoprotective medications taken by the patients after the biopsy, which might have affected the association between IAH and the progression of diabetic nephropathy.

In conclusion, aggravated AH is a histological risk factor that can predict the onset of both macroalbuminuria and eGFR <60 in patients with type 2 diabetes with normo- and microalbuminuria. Predicting the future risk of eGFR <60 through IAH will be informative, as eGFR can decrease without an increase in albuminuria. Further investigations are needed to identify new biomarkers that reflect pathological changes in diabetic nephropathy, which will lead to the establishment of novel strategies for its prevention and treatment.

Acknowledgments

We thank Ms. Noriko Nemoto of Kitasato Bio-Imaging Center for her technical assistance.

Statement of Ethics

This study was performed in accordance with the Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Committee of Kitasato University School of Medicine and Kitasato University Hospital, approval number B20-350. Requirement for individual patient informed consent was waived by the IRB because of the retrospective design of this study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Funding Sources

This research did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

Akihiko Suzuki and Tatsumi Moriya collected and analyzed the data and wrote the manuscript, including a review of the literature. Madoka Matsubara, Akinori Hayashi, and Takeshi Miyatsuka contributed to the discussions. Tatsumi Moriya is the guarantor of this work and, as such, has full access to all of the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis. This study was presented in part at the 81st Annual Scientific Meeting of the American Diabetes Association, June 25–29, 2021.

Data Availability Statement

The datasets generated and analyzed during the current study are not publicly available due to privacy or confidentiality issue, but they are available from the corresponding author on reasonable request.

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