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REVIEW ARTICLE

Nodular lesions and mesangiolysis in diabetic nephropathy

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Abstract Diabetic nephropathy is a leading cause of end-stage renal failure all over the world. Advanced human diabetic nephropathy is characterized by the presence of specific lesions including nodular lesions, doughnut lesions, and exudative lesions. Thus far, animal models precisely mimicking advanced human diabetic nephropathy, especially nodular lesions, remain to be fully established. Animal models with spontaneous diabetic kidney diseases or with inducible kidney lesions may be useful for investigating the pathogenesis of diabetic nephropathy. Based on pathological features, we previously reported that diabetic glomerular nodular-like lesions were formed during the reconstruction process of mesangiolysis. Recently, we established nodular-like lesions resembling those seen in advanced human diabetic nephropathy through vascular endothelial injury and mesangiolysis by administration of monocrotaline. Here, in this review, we discuss diabetic nodular lesions and its animal models resembling human diabetic kidney lesions, with our hypothesis that endothelial cell injury and mesangiolysis might be required for nodular lesions.

Keywords Diabetic nephropathy · Nodular lesion · Mesangiolysis · Glomerulosclerosis · Extracellular matrix · Matrix metalloproteinase

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Introduction

The number of patients with chronic kidney disease (CKD) on dialysis due to diabetic nephropathy is increasing worldwide. In Japan, the annual incidence of starting dialysis due to diabetic nephropathy has been in first place since 1998, indicating diabetic nephropathy as one of the most important causes of end-stage kidney disease. In clinical settings, insights for functional–pathological relationships in diabetic nephropathy are required for better prognosis. Generally speaking, it appears that in patients with type 2 diabetes, kidney structural changes are more heterogenous and diabetic glomerulopathy lesions are less severe than in type 1 diabetes patients with similar urine albumin levels [1]. The presence of persistent albuminuria is the hallmark of clinical diagnosis of diabetic nephropathy [1, 2]. However, some type 2 diabetic patients with microalbuminuria and proteinuria have lesions showing strikingly normal glomerular appearance or less advanced glomerular diseases than those observed in type 1 diabetes with similar urine albumin excretion [3, 4].

Characteristic pathologic changes of diabetic nephropathy are accumulation of extracellular matrix (ECM) in

glomerular and tubulointerstitial tissues, and exudative lesions [5]. Among these diabetic lesions, specific glomerular lesions in advanced human diabetic nephropathy include nodular lesions, arteriolar lesions (both efferent and afferent arterioles), and doughnut lesions. Nodular lesions are of importance for advanced diabetic nephropathy, which correlates with massive proteinuria in clinical settings. Nodular lesions were first described by Kimmelstiel and Wilson in 1936 [6]. In contrast, nodular lesions are hardly observed in experimental diabetic models showing high glucose levels without other insults. Insults in addition to glucose levels, such as endothelial cell nitric oxide synthase (eNOS) deficiency [7], integrin $\alpha 1$ deficiency [8] and the presence of Thy-1.1 nephritis [9], may be required for the formation of diabetic nodular lesions. Therefore, deeper insights into the pathogenesis of diabetic advanced lesions, including nodular lesions, are required. However, pathogenesis of these specific lesions in advanced diabetic nephropathy remains to be fully investigated. Further studies would be required to determine the pathogenesis of nodular lesions, as well as other lesions characteristic to advanced diabetic nephropathy, such as exudative lesions.

Here in this manuscript, we focus on pathogenesis and lessons from animal models of nodular lesions characteristic to advanced human glomerular lesions, both in type 1 and 2 diabetic patients.

Nodular lesions in advanced human diabetic nephropathy

Nodular lesions in human diabetic nephropathy have been reported in 10–50 % of biopsy specimens in both type 1 and type 2 diabetes [5]. Nodular lesions contain extracellular matrix components, including type IV collagen, which exists in normal glomerular structure. Electron microscopic studies revealed that nodular lesions consisted of accumulation of mesangial matrix with visible collagen fibrils, fibrils of unknown origin, small lipid particles, and cell debris [10–12]. Nodular lesions appear to develop from the central area of a stalk of a glomerular tuft, and encroach upon the surrounding capillary lumens [12]. In contrast, we previously reported that mesangiolysis might play a role in the formation of nodular lesions, with the deposition of type VI collagen in glomeruli (Fig. 1) [13, 14]. Nodular lesions possibly associated with mesangiolysis sometimes contain laminated structure. Surrounding the strongly Periodic Acid Methenamine silver (PAM)-positive materials, weakly PAM-positive materials with circumferential lamination as nodular lesions were observed (Fig. 1). Type IV collagen was mainly detected in the strongly PAM-positive area, whereas type VI collagen was detected in the weakly PAM-positive area. In this study, we examined 355 kidney specimens obtained from 327 patients with primary diabetes mellitus [13]. This report hypothesizes that: (1) the mesangiolysis is the initial lesion occurring in glomeruli in the process of diabetic nodule formation, and disturbed blood flow into glomeruli, caused by diabetic arteriolosclerosis, may be implicated in the development of the mesangiolysis; and (2) concentric compression of the lysed mesangial matrix by recanalized capillaries forms layered structures and ultimately completed diabetic nodules [13]. Hong et al. reported that diabetic patients with biopsy-proven nodular lesions had longer diabetic durations, more severe renal damage, and exhibited a poorer kidney prognosis. Furthermore, patients with nodular lesions more frequently revealed evidence of diabetic retinopathy [15]. Supporting this notion, Schwartz et al. [16] described that patients with Kimmelstiel–Wilson nodules had elevated serum creatinines and more severe overall retinopathy than those without Kimmelstiel–Wilson nodules. In contrast, kidney biopsy specimens from patients at presentation without overt clinical manifestations of glucose intolerance revealed typical nodular glomerulosclerosis, a negative deposition based on an immunofluorescence study, and neither any significant electron dense deposits nor fibrils on electron microscopy [17]. In this study, Sanai et al. concluded that diabetic nodular glomerulosclerosis occurred in patients without overt diabetes, suggesting the role of factors additional to prolonged hyperglycemia in the pathogenesis of this disorder. Interestingly, supporting this notion, nodular lesions have been also observed in membranoproliferative glomerulonephritis, amyloidosis, and light-chain deposition disease without diabetes [5]. In addition, nodular lesions are also observed in Goodpasture's syndrome [18]. Among these diseases, the strongly PAM-positive materials increased in glomeruli. However, mesangiolysis is not always observed in patients with nodular lesions with or without diabetic conditions. We previously reported nodular lesions in diabetes coexisting with mesaigiolysis or without mesangiolysis [13], although underlining mechanisms remain to be investigated. Further studies to determine mechanisms involved in the pathogenesis of nodular lesions with or without diabetes would be required.

Nodular-like lesions and mesangiolysis in an experimental model

To address the hypothesis that vascular endothelial injury and mesangiolysis are closely related to the formation of nodular lesions in diabetes, we attempted to induce nodular-like lesions resembling those seen in human patients by administration of monocrotaline [19]. In this study, marked mesangial expansion was observed in Otsuka Long-Evans

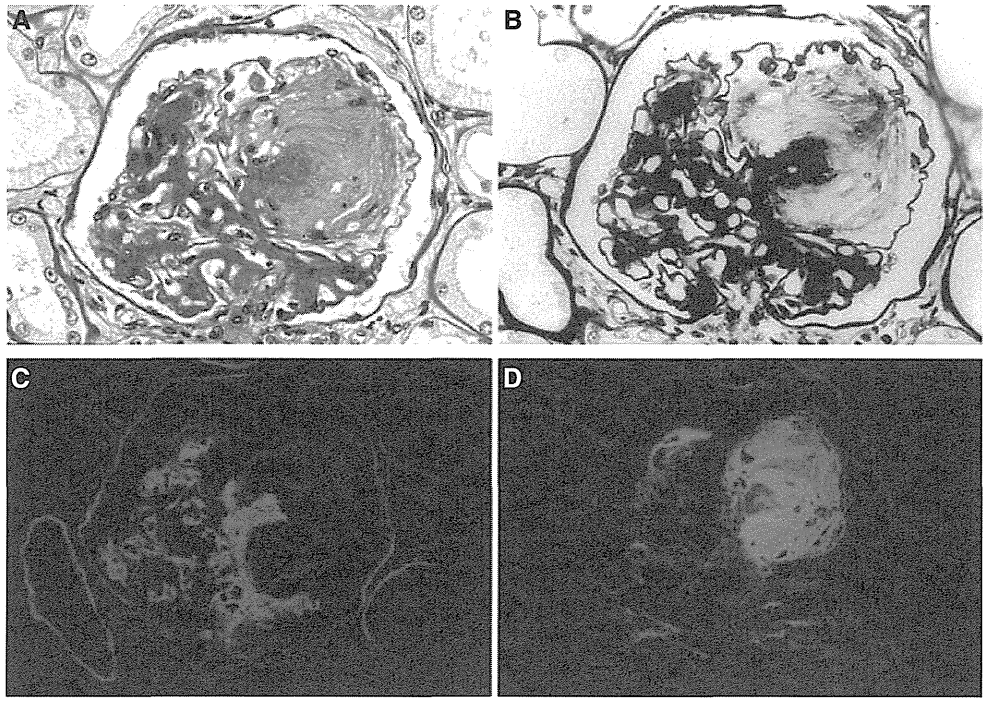

Fig. 1 Human diabetic nodular lesions by light microscopy, using serial sections. Surrounding the strongly Periodic Acid Methenamine silver (PAM)-positive materials, weakly PAM-positive materials with circumferential lamination as nodular lesions were observed (**a** Periodic Acid-Schiff (PAS) stain, ×250, **b** PAM stain, ×250). Type IV collagen was mainly detected in the strongly PAM-positive area (**c** ×250), and type VI collagen in the weakly PAM-positive area (**d** ×250). (From Ref. [14], reproduced with permission from Japanese Society of Nephrology)

Tokushima Fatty (OLETF) rats, in contrast to Long-Evans Tokushima Otsuka (LETO) rats, used as controls, after monocrotaline injection. Moreover, some glomeruli showed typical mesangiolytic lesions and nodular-like lesions in monocrotaline-treated OLETF rats at 50 weeks, concomitant with glomerular capillary endothelial cell swelling and nodular-like lesions and mesangiolysis gradually increased in monocrotaline-treated OLETF rats (Fig. 2) [19]. Matrix metalloproteinase (MMP)-2 and membrane-type 1 (MT1)-MMP proteins increased in the expanded mesangial lesions in OLETF rats as compared to LET rats. Gelatin zymography revealed an increase in 62-kDa activated MMP-2 in the culture supernatants of isolated glomeruli from OLETF rats. In situ enzymatic activity of MMP in the mesangial areas was also detected in 50-week-old monocrotaline-injected OLETF rats. The most compelling part of our study was proceeding to mesangiolysis under hyperglycemic conditions, possibly followed by the formation of nodular-like lesions. These results suggest that diabetic conditions and some toxic events associated with monocrotaline injection during the process of reconstruction from mesangiolysis may be required for the formation of nodular-like lesions resembling human ones in this model, although the lamination of nodules in this model was incomplete.

Nodular-like lesions in experimental models

The Animal Models of Diabetic Complications Consortium (AMDCC), created in 2001, describes research criteria for validating a progressive mouse model of diabetic nephropathy [20]. Especially, pathology of kidneys defines criteria consisting of four issues: (1) advanced mesangial matrix expansion ± nodular sclerosis and mesangiolysis; (2) any degree of arteriolar hyalinosis; (3) glomerular basement membrane thickening by > 50 % over baseline; and (4) tubulointerstitial fibrosis. AMDCC investigators conclude that no current model meets them all. Therefore, the criteria should be viewed as goals, rather than requirements [20].

A few reports have described experimental diabetic models resembling human nodular lesions. Inagi et al. [21] utilized megsin overexpression in a receptor for advanced glycation end products (RAGE) and inducible nitric oxide synthase (iNOS) transgenic mice. In this particular model, the triple transgenic mice overexpressing megsin, RAGE, and iNOS developed, at an early age (16 weeks), severe albuminuria and kidney damage, characterized by development of mesangial expansion, and tubulointerstitial damage, with all of the characteristics of human diabetic nephropathy. In addition, 30–40 %

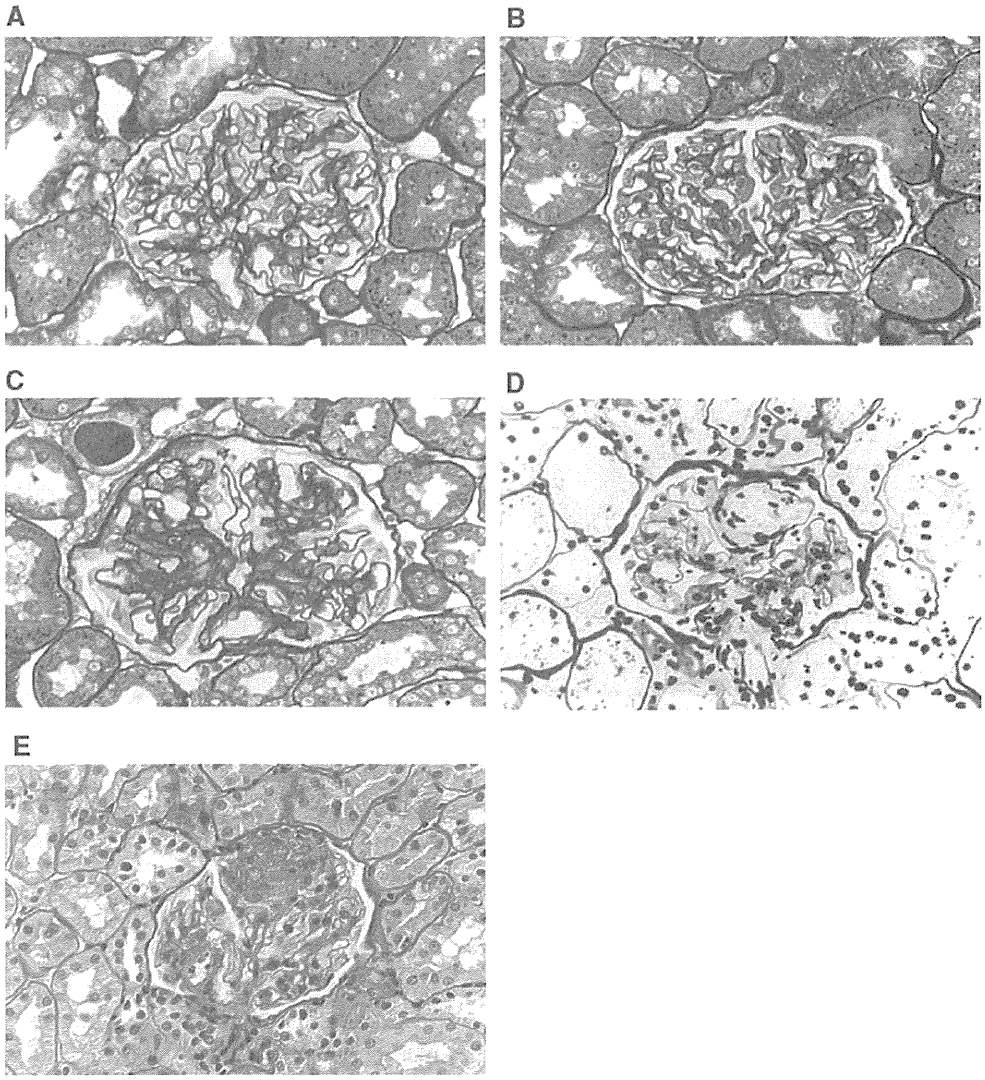

Fig. 2 Mesangiolysis and diffuse glomerulosclerosis detected in monocrotaline-injected Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Representative kidney tissues obtained from a saline-injected Long-Evans Tokushima Otsuka (LETO) rat (50 weeks **a**) and a saline-injected OLETF (50 weeks **c**). A monocrotaline-injected LETO (50 weeks **b**), and a monocrotaline-injected OLETF (50 weeks **d**, **e**) showing mesangiolysis and nodular-like lesions. **a–d** PAM stain, ×400; **e** PAS stain, ×400. (From Ref. [19], reproduced with permission from The European Renal Association, European Dialysis and Transplant Association)

of glomeruli exhibit nodule-like lesions with an increase in local oxidative stress, which may be relevant to previous reports describing the possible involvement of oxidative stress [22]. Further, endothelial nitric oxide synthase (eNOS) (−/−) mice, backcrossed to C57BLKS/J db/db mice, exhibited dramatic albuminuria, arteriolar hyalinosis, increased glomerular basement membrane thickness, mesangial expansion, mesangiolysis, and focal segmental and early nodular glomerulosclerosis by 26 weeks, although the severity of hyperglycemia was similar to C57BLKS/J db/db mice [7]. Remarkably, eNOS(−/−) C57BLKS db/db exhibited decreases in GFR to levels < 50 % of that in eNOS(+/+) C57BLKS db/db. Similarly, The $eNOS^{-/-}/lepr^{db/db}$ double-knockout mice showed significant glomerular injury, including mesangiolysis, microaneurysms, increased mesangial matrix expansion with nodular lesions (nodular glomerulosclerosis) and globally sclerotic glomeruli [23]. Further, in streptozotocin-induced eNOS knockout mice, deficiency of eNOS-derived NO causes glomerular endothelial injury in the setting of diabetes and results in overt albuminuria and glomerular mesangiolysis and nodular-like lesions in nephropathy-resistant inbred C57BL6 mice [24]. Supportingly, the level of eNO may be one of the critical determinants for whether patients with diabetes are at risk for developing nephropathy, including nodular-like lesions [25].

Molecular mechanisms possibly involved in nodular lesions

Putative promoters of progression of diabetic nephropathy include systemic blood pressure, glomerular hypertension, proteinuria, glycemic control, renin–angiotensin–aldosterone system, hyperlipidemia, obesity, dietary protein intake, smoking, oligonephropathy, and angiotensin-converting enzyme ID polymorphism [1]. In addition to these promoters, recent studies reveal that inflammatory processes may play a key role in the progression of advanced diabetic nephropathy [26, 27]. Pro-inflammatory cytokines and chemokines have been reported to contribute to the progression of advanced diabetic kidney lesions. Supporting this notion, urinary levels of monocyte chemoattractant protein (MCP)-1/CCL2, a prototype of chemokine, increased in accordance with the damage of human glomerular diffuse lesions through infiltration and activation of macrophages [27]. In addition, there was a significant correlation between urinary MCP-1 levels and the presence of nodular lesion and mesangiolysis. Moreover, the mitogen-activated protein kinase (MAPK) phosphorylation, deeply involved in production and signal transduction of pro-inflammatory cytokines and chemokines, contributes to human diabetic nephropathy, including the presence of nodular lesions [28]. In particular, extracellular signal-regulated kinase (ERK) may be distinctly involved in glomerular lesions in human diabetic nephropathy. Interestingly, Thy-1.1 nephritis aggravated diabetic nephropathy, leading to the formation of nodular-like lesions [9]. Mesangiolysis and macrophage activation, accompanied by mesangial damage, are essential in this particular model. Collectively, the interaction between kidney resident cells and infiltrating cells enhances the synthesis matrix protein, cytokines/chemokines and pro-fibrotic growth factors, which may promote and escalate chronic inflammatory processes, thereby perpetuating kidney fibrosis, including advanced diabetic nephropathy [29–32].

We previously reported that diabetic glomerular lesions were formed during the reconstruction process of mesangiolysis. In this setting, matrix metalloproteinase (MMP)-2, which is activated from proMMP-2 by membrane-type (MT)-MMP, was produced and activated in glomeruli through the progression of diabetic nephropathy in a type 2 diabetic model, OLETF rats [19]. These findings suggest matrix turnover regulated by MMPs, and their modulators may have some effect on the remodeling of the glomerular matrix in diabetic nephropathy.

In close relation to inflammatory processes, advanced glycation end-products (AGEs) seem to play a central role in the progression of diabetic nephropathy [33]. AGEs are localized most notably in nodular lesions, suggesting that advanced glycations may play a role in the progression of diabetic nephropathy through impairment of the assembly of matrix proteins in vivo. Because type V and type VI collagens are the major components of nodular lesions, increases in these interstitial and fibril or microfibril collagens may contribute to the formation of wider strands in the mesangial matrix of a nodular lesion. As no metalloprotease that is specific for type VI collagen has been identified thus far, AGE formation might occur preferentially in type VI collagen-rich nodular lesions, which are sites of slow turnover [33]. These results may be relevant to our previous observation [13, 19, 22] (Fig. 3).

Further, endothelial dysfunction that accompanies a diabetic state may cause advanced diabetic nephropathy, including nodular-like lesions [7, 23–25]. Endothelial damage induced by eNOS deficiency may be responsible for an uncoupling of the VEGF-eNO axis, resulting in increased levels of VEGF and excessive endothelial cell proliferation, thereby coupled with altered autoregulation consequent to the development of preglomerular arteriolar disease [25]. Therefore, together with our previous studies in humans [13] and an experimental model [19], insults resulting in endothelial damage may be one of the important determinants for whether patients with diabetes are at risk for developing advanced nephropathy.

More recently, novel mechanisms involved in anti-inflammatory effects in the progression of diabetic nephropathy have been reported. Podocyte-specific overexpression of human angiotensin-converting enzyme 2 attenuated diabetic nephropathy in mice [34]. Further, hepatocyte growth factor gene therapy enhanced renal expression of stromal-cell-derived factor-1 and was subsequently associated with an increased number of bone-marrow-derived cells getting into the injured kidneys in db/db mice [35]. Interestingly, these cells were mainly monocyte-derived macrophage, which may contribute to kidney tissue repair and regeneration. In contrast, cholecystokinin suppressed the activation of macrophage and expression of pro-inflammatory genes in diabetic kidneys [36].

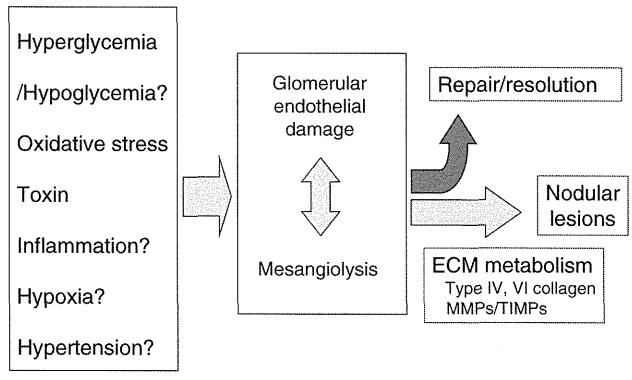

Fig. 3 Possible mechanism in nodular lesions associated with mesangiolysis

Collectively, further studies would be required to determine the precise mechanisms involved in formation and progression of nodular lesions and mesangiolysis, including origin and roles of macrophages, key players in the progression of advanced diabetic lesions.

Concluding remarks and future directions

We have introduced the pathological features and experimental models of diabetic nodular lesions. Because diabetic nodular lesions are of importance in the progression of advanced human diabetic nephropathy, it is essential that the pathogenesis of nodular lesions and mesangiolysis be elucidated in near future. This may provide a key not only for better understanding, but for the therapeutic benefit of diabetic nephropathy.

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ORIGINAL ARTICLE

Clinical impact of albuminuria and glomerular filtration rate on renal and cardiovascular events, and all-cause mortality in Japanese patients with type 2 diabetes

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Abstract
Background The number of patients suffering from diabetic nephropathy resulting in end-stage kidney disease is increasing worldwide. In clinical settings, there are limited data regarding the impact of the urinary albumin-to-creatinine ratio (UACR) and reduced estimated glomerular filtration rate (eGFR) on renal and cardiovascular outcomes and all-cause mortality.
Methods We performed a historical cohort study of 4328 Japanese participants with type 2 diabetes from 10 centers. Risks for renal events (requirement for dialysis or transplantation, or half reduction in eGFR), cardiovascular events (cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke), and all-cause mortality were assessed according to UACR and eGFR levels.
Results During follow-up (median 7.0 years, interquartile range 3.0–8.0 years), 419 renal events, 605 cardiovascular events and 236 deaths occurred. The UACR levels increased the risk and the adjusted hazard ratios for these three events. In addition to the effects of UACR levels, eGFR stages significantly increased the adjusted hazard ratios for renal events and all-cause mortality, especially in

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patients with macroalbuminuria. Diabetic nephropathy score, based on the prognostic factors, well predicted incidence rates per 1000 patient/year for each event.
Conclusions Increased UACR levels were closely related to the increase in risks for renal, cardiovascular events and all-cause mortality in Japanese patients with type 2 diabetes, whereas the association between high levels of UACR and reduced eGFR was a strong predictor for renal events.

Keywords Diabetic nephropathy · Chronic kidney disease · Albuminuria · Cardiovascular disease · Mortality · Glomerular filtration rate

Introduction

Diabetic nephropathy is a leading cause of end-stage kidney (renal) disease (ESKD or ESRD) worldwide [1]. In addition, cardiovascular diseases and deaths increase in patients with diabetic nephropathy before and after dialysis [2–4]. Therefore, to determine and manage risk factors for progression of renal and cardiovascular outcomes and mortality is of importance to prolong the life expectancy of diabetic patients.

A high urinary albumin-to-creatinine ratio (UACR) and low estimated glomerular filtration rate (eGFR) have been believed to be predictors for diabetic ESKD and death [5–7]. Kidney Disease Improving Global Outcomes (KDIGO) provided a new classification for chronic kidney disease (CKD) by adding stages that stratified urinary albumin excretion as well as eGFR and emphasizing clinical diagnosis [8]. This new classification, mainly based on the collaborative meta-analysis of general population cohorts [8], has shed light on prognosis assigned by clinical diagnosis, stage, and other key factors relevant to renal and cardiovascular outcomes. However, the clinical impact of UACR levels in combination with eGFR on outcomes in Japanese patients with diabetic nephropathy needs to be confirmed. Therefore, deeper clinical insights of UACR along with GFR are required to provide a key for the pathogenesis and outcomes of progressive renal complications, and associated cardiovascular events in type 2 diabetic patients.

Here we examined the prognostic value of UACR and eGFR for renal events, cardiovascular events and all-cause mortality in Japanese patients with type 2 diabetes. Furthermore, we proposed a diabetic nephropathy score for predicting prognosis in diabetic patients.

Subjects and methods

Subjects

This study was a historical cohort consisting of 4814 Japanese patients with type 2 diabetes who were treated by trained physicians at 10 centers between 1985 and 2010. Four hundred and fifty-nine patients were excluded because of age <18 ($n = 6$), follow-up <1 year ($n = 151$) and no measurement of urinary albumin or HbA1c or blood pressure (BP) ($n = 329$), leaving 4328 Japanese patients to be enrolled in this study. Patients with secondary diabetes, renal transplantation or dialysis were also excluded.

This study was conducted according to the ethical guidelines for epidemiological research designed by the Japanese Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour, and Welfare (http://www.lifescience.mext.go.jp/files/pdf/n796_01.pdf).

The study design was included in a comprehensive protocol of retrospective study at the Division of Nephrology, Kanazawa University Hospital approved by Kanazawa University ethical committee (approval number 815).

Follow-up and assessments

Type 2 diabetes was defined according to the Japan Diabetes Society (JDS) criteria [9]. In this historical cohort study, UACR and eGFR were also determined. Measurement of UACR, by a turbidimetric immunoassay at each laboratory, was performed on spot urine samples at baseline. Serum creatinine was measured at baseline, at subsequent yearly intervals, and at the end of follow-up. Serum and urinary concentrations of creatinine were measured by an enzymatic method, and eGFR was estimated using the equation proposed by the Japanese Society of Nephrology [10]. Both UACR and serum creatinine were measured at local laboratories. At each study visit, blood pressure (BP) was measured in the sitting position. Hypertension was defined as BP $\geq$ 140/90 mmHg or

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current use of antihypertensive drugs. Non-fasting blood samples were obtained for measurements of HbA1c and lipid levels at local laboratories. HbA1c was measured and standardized by the JDS (normal range 4.3–5.8 %) and certified by the US National Glycohemoglobin Standardization Program (National Glycohemoglobin Standardization Program, NGSP; NGSP = JDS + 0.4) [9].

UACR and GFR categories

Based on the new classification of CKD [8], the albuminuria category was classified at baseline as normoalbuminuria (<30 mg/g), microalbuminuria (≥30 and <300 mg/g), and macroalbuminuria (≥300 mg/g). In addition, baseline eGFR levels were divided into six categories: ≥90, 60–89, 45–59, 30–44, 15–29 and <15 ml/min per 1.73 m^2. Patients examined in this study were categorized and assessed based on the above classifications.

Outcomes

The main outcomes of this study were renal events (requirement for dialysis or transplantation, or half reduction in eGFR), cardiovascular events (cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke), and all-cause mortality death. These conditions corresponded to the International Classification of Diseases, 11th version (http://www.who.int/classification/icd/en/). Definitions for nonfatal myocardial infarction and nonfatal stroke are given elsewhere [11]. Patients were referred to cardiologists, neurosurgeons, neurologists or else to confirm diagnoses. Only the first event of the relevant outcome type was included in each analysis and the last day of the observation period was also noted if there were no incidences.

Statistical analysis

Data are expressed as mean ± SD or median (interquartile range). Incidence rates of renal events, cardiovascular events, and all-cause death for different categories were calculated. Cox proportional hazards analysis was used to compute hazard ratios and 95 % confidence intervals (CI) to assess the impact of albuminuria and eGFR on the outcomes by using the group with eGFR ≥60 ml/min per 1.73 m^2 and/or the group with normoalbuminuria (<30 mg/g) as the reference [8]. In multivariate analysis, adjustment for risk factors for renal events, cardiovascular events, or all-cause mortality included age, gender, HbA1c, and systolic BP. A p value <5 % was considered significant. p values for trend tests examined whether UACR and eGFR levels were associated with increased hazard ratios. Trend tests across increasing risks for renal, cardiovascular events and all-cause mortality are stratified by factors for diabetic nephropathy score.

All analyses were performed with the statistical software package SPSS (SPSS Japan, Tokyo, Japan).

Results

Baseline characteristics

Table 1 shows the baseline characteristics of patients examined in this study. The 4328 patients were distributed according to CKD stage and were followed until the onset of the first event or the end of the observation period (Table 1).

Incidence of numbers of patients of each event

During a median follow-up of 7.0 years (interquartile range 3.0–8.0 years), 419 renal events, 605 cardiovascular events and 236 deaths occurred, which were stratified by stages of renal function and levels of UACR with each event (Table 2). The incidence rates of each outcome per 1000 person-years were 19.8 for renal events, 23.3 for cardiovascular events and 8.4 for all-cause mortality. The incidence of each event increased with worsening of UACR levels and eGFR stages. Of importance, high incidence rates were noted in patients with macroalbuminuria plus reduced eGFR, especially for renal events (Table 2).

Risk for renal events, cardiovascular events, and all-cause mortality stratified by albuminuria and eGFR

Risks for renal events, cardiovascular events and all-cause mortality were evaluated by Cox proportional hazards analysis. The estimates were adjusted for age, gender, HbA1c, and systolic BP. The adjusted hazard ratios for renal events were 3.21 (95 % CI 2.31–4.47) for microalbuminuric patients and 21.86 (95 % CI 16.15–29.59) for macroalbuminuric patients as compared to normoalbuminuric patients as reference. Similarly, the adjusted hazard ratios for cardiovascular events and all-cause mortality were 1.38 (95 % CI 1.14–1.67) and 1.37 (95 % CI 0.99–1.89) for microalbuminuric patients and 2.05 (95 % CI 1.61–2.58) and 3.60 (95 % CI 2.53–5.20) for macroalbuminuric patients as compared to reference, respectively. Interestingly, UACR levels had the most significant impact on renal events. In addition to the effects of UACR levels, eGFR stages significantly increased the adjusted hazard ratios for renal events in patients with macroalbuminuria (Table 3). In Table 3, hazard ratios for cardiovascular events increased in patients with a higher UACR. In

Table 1 Baseline characteristics of participants ($n = 4328$)

Variable	All	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	p For trend
N	4328	2679	1115	534	
Age (years; mean [SD])	60.2 (11.6)	59.5 (11.4)	61.9 (11.5)	59.8 (12.0)	<0.001
Male (*n* [%])	2546 (58.8)	1531 (57.1)	656 (58.8)	359 (67.2)	<0.001
Kidney factors					
UACR (mg/g; median [IQR])	18.2 (8.6–66.6)	10.2 (6.5–16.4)	66.6 (42.6–121.3)	994.8 (518.5–2272.5)	<0.001
eGFR (ml/min/1.73 m^2; mean [SD])	77.0 (25.9)	81.3 (23.8)	76.4 (25.3)	56.9 (28.0)	<0.001
eGFR ≥90 (*n* [%])	1201 (27.7)	839	297	65	
eGFR 60–89 (*n* [%])	2051 (47.4)	1371	530	150	
eGFR 45–59 (*n* [%])	642 (14.8)	345	174	123	
eGFR 30–44 (*n* [%])	311 (7.2)	109	100	102	
eGFR 15–29 (*n* [%])	117 (2.7)	15	14	88	
eGFR <15 (*n* [%])	6 (0.1)	0	0	6	
BP (mmHg)					
Systolic BP (mean [SD])	131.0 (18.6)	127.2 (16.6)	134.2 (18.4)	143.0 (22.0)	<0.001
Diastolic BP (mean [SD])	74.3 (18.0)	73.3 (20.7)	74.8 (11.8)	78.1 (13.1)	<0.001
Other major risk factors					
HbA1c (%; mean [SD])	7.6 (1.7)	7.5 (1.7)	7.8 (1.7)	7.9 (1.8)	<0.001
Total cholesterol (mg/dL; mean [SD])	205.2 (35.9)	205.3 (34.1)	202.2 (33.9)	214.2 (50.2)	0.925
Body mass index (kg/m^2; mean [SD])	25.3 (4.2)	25.1 (4.2)	25.5 (4.2)	25.4 (4.8)	0.098

Table 2 Number of patients and incidence rates of each outcome stratified by stages of eGFR and albuminuria

UACR	eGFR (ml/min/1.73 m^2)					
	>90	60–89	45–59	30–44	15–29	<15
Renal events (RRT or halving reduced eGFR)						
Normoalbuminuria	58 (4.2)		4 (2.3)	3 (6.9)	1 (21.3)	0
Microalbuminuria	31 (17.2)	41 (13.1)	15 (18.0)	10 (21.0)	1 (25.6)	0
Macroalbuminuria	20 (59.5)	62 (87.7)	56 (126.7)	54 (193.5)	61 (309.6)	2 (250.0)
Cardiovascular events						
Normoalbuminuria	229 (16.2)		40 (23.1)	14 (33.0)	1 (18.9)	0
Microalbuminuria	31 (16.1)	95 (28.7)	33 (32.6)	28 (56.1)	2 (43.5)	0
Macroalbuminuria	7 (17.2)	41 (44.6)	30 (44.3)	30 (64.2)	23 (57.9)	1 (100.0)
All-cause mortality						
Normoalbuminuria	70 (4.7)		26 (13.8)	4 (8.2)	4 (67.8)	0
Microalbuminuria	11 (5.4)	32 (8.9)	13 (11.9)	5 (8.5)	6 (117.6)	0
Macroalbuminuria	6 (14.4)	13 (12.3)	19 (24.6)	13 (23.6)	12 (26.8)	2 (142.9)

Number of patients (incidence rates per 1000 person-years)

RRT renal replacement therapy

addition, our results showed that there was a slight increase in the hazard ratios of cardiovascular events based on UACR levels plus co-existing reduced eGFR, especially in patients with microalbuminuria based on p for trend. In contrast, all-cause mortality was strongly associated with reduced eGFR <30 ml/min per 1.73 m^2, and the presence of macroalbuminuria even with preserved eGFR. The present study also revealed that normoalbuminuric renal insufficient diabetic patients did not have relatively poor outcomes for renal events. Table 4 highlights the impact of low GFR and/or UACR on three distinct outcomes.

The clinical significance of diabetic nephropathy score in predicting the prognoses of renal events, cardiovascular events, and all-cause mortality

Considering the results of univariable and multivariable analyses, weighted arbitrary scores were allocated to each

Table 3 Hazard ratios based on CKD stages for each outcome

UACR	eGFR (ml/min/1.73 m^2)						*p* for trend (eGFR)
	>90	60–89	45–59	30–44	15–29	<15	
Renal events (RRT or halving reduced eGFR)							
Normoalbuminuria	1.00 (Reference)	1.00 (Reference)	0.69 (0.24–1.98)	1.83 (0.53–6.31)	11.59 (1.43–93.78)	NA	0.85
Microalbuminuria	3.31 (2.07–5.28)	3.04 (1.98–4.68)	3.36 (1.63–6.93)	3.10 (1.41–6.83)	3.60 (0.42–31.28)	NA	0.60
Macroalbuminuria	11.14 (5.87–21.17)	15.64 (10.30–23.74)	33.37 (20.58–50.91)	41.36 (25.09–68.16)	71.58 (40.41–126.80)	NA	<0.01
p for trend (albuminuria)	<0.01	<0.01	<0.01	<0.01	0.06	NA	
Cardiovascular events							
Normoalbuminuria	1.00 (Reference)	1.00 (Reference)	1.05 (0.73–1.49)	1.30 (0.74–2.28)	0.42 (0.06–3.06)	NA	0.46
Microalbuminuria	1.01 (0.69–1.49)	1.48 (1.15–1.90)	1.33 (0.89–2.00)	1.85 (1.20–2.85)	0.47 (0.11–1.97)	NA	0.04
Macroalbuminuria	1.28 (0.56–2.94)	2.10 (1.46–3.02)	1.85 (1.23–2.78)	2.37 (1.55–3.63)	2.09 (1.26–3.45)	12.76 (0.95–171.19)	0.20
p for trend (albuminuria)	0.81	<0.01	0.09	0.45	0.17	NA	
All-cause mortality							
Normoalbuminuria	1.00 (Reference)	1.00 (Reference)	1.67 (1.02–2.74)	1.22 (0.43–3.46)	8.19 (2.65–25.34)	NA	<0.01
Microalbuminuria	1.51 (0.78–2.95)	1.44 (0.92–2.24)	1.22 (0.63–2.35)	0.84 (0.31–2.26)	8.36 (2.81–24.90)	NA	0.04
Macroalbuminuria	4.37 (1.70–11.24)	1.92 (0.97–3.79)	4.84 (2.72–8.62)	4.09 (2.00–8.34)	6.16 (2.80–13.56)	70.57 (3.65–1363.68)	0.06
p for trend (albuminuria)	0.01	0.01	0.01	0.02	0.80	NA	

The estimates are adjusted for age, gender, HbA1c, systolic BP

RRT renal replacement therapy, *NA* not available

Table 4 Hazard ratios based on levels of UACR and eGFR for each outcome

UACR	eGFR (ml/min/1.73 m^2)		
	>60	30–59	<30
Renal events (RRT or halving reduced eGFR)			
Normoalbuminuria	1.00 (Reference)		49.82 (29.9–83.0)
Microalbuminuria	3.26 (2.34–4.55)		
Macroalbuminuria	13.6 (9.3–20.0)	33.0 (22.7–48.2)	
Cardiovascular events			
Normoalbuminuria	1.00 (Reference)		1.54 (1.00–2.39)
Microalbuminuria	1.40 (1.16–1.69)		
Macroalbuminuria	1.90 (1.36–2.65)	2.09 (1.54–2.84)	
All-cause mortality			
Normoalbuminuria	1.00 (Reference)		7.08 (4.16–12.05)
Microalbuminuria	1.30 (0.93–1.81)		
Macroalbuminuria	2.34 (1.35–4.04)	4.59 (2.90–7.25)	

The estimates are adjusted for age, gender, HbA1c, systolic BP

selected variable on the basis of each odds ratio (OR), and we defined a summation of scores as a new risk scoring system as the diabetic nephropathy score. We evaluated the diabetic nephropathy score for predicting the prognoses of renal events, cardiovascular events, and all-cause mortality. Each prognostic factor has a score and the maximum score is 6—microalbuminuria = 1, macroalbuminuria = 2, eGFR <45 ml/min per 1.73 m^2 = 1, age ≥60 years = 1, systolic BP >130 mmHg = 1, and HbA1c (NGSP) ≥6.9 % = 1. This score put stress on amounts of UACR. Importantly, this simple score well predicted the incidence rates per 1000 patient/year for each event (Table 5).

Discussion

In this study we examined the clinical impact of UACR as well as the evaluation of GFR on outcomes in diabetic patients. We now report that increased urinary albumin excretion was strongly associated with risks for renal

Table 5 Diabetic nephropathy score reflects diabetic outcomes

Score	Renal events (RRT or halving reduced eGFR)				Cardiovascular events				All-cause mortality			
	Number of patients	Number of incidents	Rate per 1000 patient-years	95 % CI	Number of patients	Number of incidents	Rate per 1000 patient-years	95 % CI	Number of patients	Number of incidents	Rate per 1000 patient-years	95 % CI
0	204	0	0.0		204	2	1.4	0.2–5.1	204	0	0.0	
1	902	18	3.1	1.9–5.0	954	58	9.7	7.4–12.6	954	13	2.1	1.1–3.6
2	1228	54	6.9	5.2–9.0	1,310	174	21.4	18.4–24.9	1310	71	8.1	6.3–10.3
3	952	96	16.6	13.4–20.0	1,017	161	26.2	22.3–30.6	1017	62	9.4	7.2–12.0
4	471	118	46.6	38.5–56.0	532	116	39.0	32.2–46.8	532	45	13.4	9.8–18.0
5	213	93	103.8	83.8–127.0	238	71	60.5	47.3–76.3	238	35	25.5	17.7–35.4
6	61	40	216.2	154.5–294.0	73	23	80.4	51.0–120.7	73	10	28.1	13.5–51.7
p for trend			<0.001				<0.001				<0.001	

events, cardiovascular events and deaths in Japanese patients with type 2 diabetes. Of note, eGFR stages significantly increased the adjusted hazard ratios for renal events, especially when co-existing with macroalbuminuria, while patients with normoalbuminuria had relatively low risks for renal events. All-cause mortality was strongly associated with reduced eGFR <30 ml/min per 1.73 m^2 and the presence of macroalbuminuria even with preserved eGFR. However, the association between normoalbuminuria and reduced eGFR showed relatively low risks for cardiovascular events in the cohort of the Japanese population with type 2 diabetes. These findings suggested that diabetic patients with macroalbuminuira and low GFR had risks for adverse outcomes, even though UACR levels and eGFR had distinct clinical impacts on each event, respectively. Finally, the diabetic nephropathy score based on our present study may be useful for predicting the prognoses of outcomes in diabetic patients.

The present study has clearly shown that renal insufficiency plus the presence of macroalbuminuria accelerated risks for adverse outcomes, especially renal events. Recently, KDIGO reported the definition, classification and prognosis of CKD based both on estimated GFR and urinary levels of albumin excretion, emphasizing that a decrease in GFR as well as macroalbuminuria is important for renal outcomes of CKD [8]. In addition, the Action in Diabetes and Vascular disease: preterAx and diamicroN-MR Controlled Evaluation (ADVANCE) study reported that reduced eGFR with macroalbuminuria was associated with a higher risk for renal events [6]. Interestingly, the Casale Monferrato study revealed that macroalbuminuira was the main predictor of mortality, independently of both eGFR and cardiovascular risk factors [12]. In contrast, reduced eGFR did not increase the adjusted hazard ratio for renal events even in patients with microalbuminuria. This may be partly because the number of microalbuminuric patients with reduced GFR having renal events was relatively small as shown in Table 2. Collectively, these findings suggest that the assessment of macroalbuminuria as well as levels of eGFR may enable us to predict high risk for renal events.

The association between UACR and reduced eGFR showed relatively low risks for cardiovascular events, even though the incidence rate of cardiovascular events was 23.3, which was almost comparable to that observed in the Japan Diabetes Complications Study (JDCS) [13]. Our results also demonstrated that UACR was closely associated with cardiovascular events in patients with eGFR 60–89 ml/min per 1.73 m^2 and that reduced eGFR was important in microalbuminuric patients based on *p* values for trend. Of note, Yokoyama et al. recently reported that the risk for cardiovascular events was associated with progression of UACR stage in type 2 Japanese diabetic

patients [14]. In contrast, reduced eGFR was a high risk for developing cardiovascular endpoints (cardiovascular death, new admissions due to to angina, myocardial infarction, stroke, revascularization or heart failure) and all-cause mortality independent of UACR [15]. Interestingly, the Second Nord-Trøndelag Health (HUNT II) study [16] reported that reduced eGFR with higher UACR was associated with a higher risk for cardiovascular events. This discrepancy compared to our present study may be partly because the number of patients with cardiovascular events in the present study was relatively small as shown in Table 2. Further studies will be required to examine this discrepancy.

This study also revealed that normoalbuminuric renal insufficient diabetic patients did not have relatively poor renal outcomes. In fact, the percentage of diabetic patients with normoalbuminuria and low eGFR is supposed to be relatively common in clinical settings. In this aspect, Yokoyama et al. [17] described that the proportion of subjects with low eGFR (<60 ml/min per 1.73 m^2) and normoalbuminuria was 11.4 % of type 2 diabetic patients examined (262/2,298). Supporting our notion, Rigalleau et al. [18] reported that risk for renal progression in such patients with type 1 or type 2 diabetes is lower. On the contrary, all-cause mortality, not cardiovascular events, was strongly associated with reduced eGFR <30 ml/min per 1.73 m^2 in normoalbuminuric diabetic patients in this present study. Supporting this notion, hazard ratios for all-cause mortality as well as cardiovascular mortality increased in normoalbuminuric diabetic patients with low GFR [19]. The FIELD study also revealed that normoalbuminuric patients with eGFR 30–59 ml/min per 1.73 m^2 had a higher risk of cardiovascular events, cardiovascular death, non-coronary heart disease deaths, death from any cause than normoalbuminuric patients with eGFR ≥60 ml/min per 1.73 m^2 [7]. Interestingly, in the ADVANCE study, patients with normoalbuminuria and eGFR <60 ml/min/1.73 m^2 had a 3.95-fold higher risk for renal events, a 1.33-fold higher risk for cardiovascular events and a 1.85-fold higher risk for cardiovascular death [6]. In contrast, Vlek et al. [20] reported that eGFR <60 ml/min per 1.73 m^2 without UACR mainly influenced the risk of vascular events (hazard ratio 1.50; 1.05–2.15), but did not affect all-cause mortality. Furthermore, in type 2 diabetic patients, eGFR provided no further information for all-cause mortality and cardiovascular mortality in normoalbuminuric patients [14]. Therefore, further studies are needed to determine renal outcomes as well as all-cause mortality in normoalbuminuric diabetic patients with low eGFR.

We proposed a novel diabetic nephropathy score to predict incidence rates per 1000 patient/year for each event. To date, few studies have addressed individual prognostic factors/scores to predict outcomes of diabetic complications in clinical settings. Couchoud et al. [21] reported development and validation of a prognostic score for 6-month mortality in elderly patients starting dialysis for ESKD. Nine risk factors were selected and points assigned for the score were body mass index <18.5 kg/m^2 (2 points), diabetes (1 point), congestive heart failure stages III to IV (2 points), peripheral vascular disease stages III to IV (2 points), dysrhythmia (1 point), active malignancy (1 point), severe behavioral disorder (2 points), total dependency for transfers (3 points) and unplanned dialysis (2 points). These scores effectively predict short-term prognosis among elderly patients, in which approximately 20 % of the patients had diabetic nephropathy. In contrast to this previous study, our simple prognostic scoring system may clearly predict cardiovascular events and all-cause mortality as well as renal events for patients of any age. Even though validation of this score system will be required for other cohorts, this system seems simple and useful for predicting clinical aspects.

To date, UACR levels and reduced eGFR have independently been reported to predict cardiovascular and real outcomes in diabetes [6]. Previously, diabetic patients with microalbuminuria/macroalbuminuira had a risk for adverse outcomes, including cardiovascular events, cardiovascular death, and renal events as reported by the ADVANCE study [6]. Importantly, the present study, consisting of 4328 Japanese patients with type 2 diabetes, was critically different from the ADVANCE study in terms of (1) being a historical cohort study consisting of 10 centers, (2) longer observation period (median 7.0 years), (3) including the assessment of all-cause mortality, (4) including assessment of each event based on the new classification CKD stages, and (5) providing a diabetic nephropathy score to predict the prognoses of renal events, cardiovascular events, and all-cause mortality. Therefore, our present study further revealed the clinical significance of UACR and eGFR on adverse outcomes in diabetic patients.

There are several limitations to this study. First, the lack of histologically proven diabetic nephropathy should be discussed, even though diabetic nephropathy is clinically diagnosed by the presence of microalbuminuria. Second, the low incidence of cardiovascular events may result in a relatively weak statistical power. Furthermore, the lack of data regarding whether enrolled patients have predisposing cardiovascular diseases must be considered. However, this multicenter observational study of 4328 diabetic patients over 7 years may strengthen the present results and increase the accuracy of risk estimation and establishment of a prognostic diabetic nephropathy score.

In conclusion, these results conclude that the presence of microalbuminuria/macroalbuminuria is closely related to the increase in risks for adverse outcomes in Japanese diabetic patients, whereas the association between

macroalbuminuria and reduced eGFR was a strong predictor for renal events. Further studies will be required to validate the prognostic factors and related diabetic nephropathy score by using other cohorts together with future perspectives.

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Conflict of interest H. Makino is a consultant for AbbVie, Astellas and Teijin, receives speaker honoraria from Astellas, MSD, Takeda, and Tanabe Mitsubishi, and receives grant support from Astellas, Daiichi Sankyo, Dainippon Sumitomo, MSD, Novo Nodisk and Takeda.

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REVIEW ARTICLE

Kidney lesions in diabetic patients with normoalbuminuric renal insufficiency

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Abstract Diabetic nephropathy is a leading cause of end-stage renal disease in Japan. Microalbuminuria has been considered as the first clinical sign of diabetic nephropathy. However, recent studies demonstrated that normoalbuminuric renal insufficiency is not uncommon for diabetic patients, especially in type 2 diabetes. Although the pathogenesis of normoalbuminuric renal insufficiency in diabetic nephropathy remains to be fully elucidated, distinct clinical and pathological features of diabetic patients with this finding have been reported as compared to those in diabetic patients with a typical clinical course. In type 1 diabetes, more advanced glomerular lesions were found in patients with normoalbuminuric renal insufficiency than in patients with normoalbuminuric preserved renal function. In contrast, disproportionately advanced tubulointerstitial and vascular lesions, despite minor diabetic glomerular lesions, which denote the presence of diabetic kidney lesions as well as nephrosclerosis, were likely to be related to the development of normoalbuminuric renal insufficiency in some type 2 diabetic patients. In addition, long-term outcomes of diabetic patients with normoalbuminuric renal insufficiency remain controversial. Further studies to gain a better understanding of the structural–functional relationships and natural history of diabetic patients with normoalbuminuric renal insufficiency may improve the benefits of therapeutic interventions for diabetic nephropathy.

Keywords Diabetic nephropathy · Normoalbuminuric renal insufficiency · Kidney lesions · Nephrosclerosis · Comprehensive medicine · Humans

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Introduction

Diabetic nephropathy is a leading cause of end-stage renal disease in Japan [1]. The percentage of patients with diabetic nephropathy was 44.1 % among new dialysis patients, and 37.1 % among all dialysis patients at the end of 2012 based on the latest annual report of the Japanese Society for Dialysis Therapy.

Albuminuria and glomerular filtration rate (GFR) are recommended for use as clinical markers of diabetic nephropathy, and microalbuminuria has been considered as the first clinical sign of diabetic nephropathy [2–5]. However, recent studies demonstrated that reduction in GFR may precede the development of microalbuminuria in some diabetic patients [6–14].

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On the other hand, pathological markers of diabetic nephropathy are complicated because a variety of renal lesions can be found in diabetic nephropathy. In addition, factors such as obesity, hypertension, dyslipidemia, and aging, which are frequent complications in type 2 diabetes, cause a wide variety of pathological changes [15–17]. Currently, renal biopsy is not always applicable for diabetic patients with a typical clinical course. The latest committee report by the Japan Renal Biopsy Registry (J-RBR) and the Japan Kidney Disease Registry (J-KDR) indicated that the percentage of diabetic nephropathy was 5.1 % (376/7442) in the pathological diagnoses as classified by pathogenesis in J-RBR 2009 and 2010 [18]. Therefore, the structural–functional relationships of diabetic nephropathy remain to be fully investigated.

Previously, Takazakura et al. [19] reported the clinical factors related to the development and progression of renal lesions in diabetic nephropathy by evaluation of serial renal biopsies or autopsy. In this study, significant relationships were found between progression of diabetic glomerulosclerosis and clinical factors such as the control of blood glucose, type of diabetes, age at onset, type of treatment, and degree of obesity. Subsequently, we conducted a long-term retrospective study to evaluate the structural–functional relationships and prognostic impacts of clinicopathological parameters for renal events, cardiovascular events, and all-cause mortality among 260 Japanese type 2 diabetic patients with biopsy-proven diabetic nephropathy [20]. Our study suggested that the characteristic pathological lesions and macroalbuminuria (severe proteinuria) were closely related to the long-term outcomes of diabetic nephropathy in type 2 diabetes. Based on our results, it is reasonable to predict renal prognosis of diabetic nephropathy by a combination of clinical and pathological parameters. Furthermore, we speculate that the evaluation of renal pathology provides a key note to have deeper insights for 'Comprehensive Medicine in Humans' including renal events and cardiovascular events in patients with diabetic nephropathy.

Here, we focus on the clinical characteristics, renal lesions, and outcomes of diabetic nephropathy with normoalbuminuric renal insufficiency, and describe future perspectives for clinical research on diabetic nephropathy.

Prevalence of normoalbuminuric renal insufficiency in diabetes

The prevalence of normoalbuminuria among type 1 diabetic patients with low GFR (<60 ml/min/1.73 m^2) was 23.6 % (21/89) in the Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) study conducted in 1982–2006 [6].

In contrast, the prevalence of normoalbuminuria among type 2 diabetic patients with low GFR (<60 ml/min/1.73 m^2) was 32 % in the Developing Education on Microalbuminuria for Awareness of Renal and Cardiovascular Risk in Diabetes (DEMAND) study conducted in 2002–2005 [7], 35.1 % (60/171) in the Third National Health and Nutrition Examination Survey (NHANES III) conducted in 1988–1994 [8], 50.8 % (575/1132) in the United Kingdom Prospective Diabetes Study (UKPDS)-74 study conducted in 1977–1991 [9], 51.8 % (262/506) in the Japan Diabetes Clinical Data Management (JDDM) study conducted in 2004–2005 [10], 55.0 % (506/920) in the National Evaluation of the Frequency of Renal Impairment cO-existing with Non-insulin-dependent diabetes (NEFRON) study conducted in 2005 [11], 56.5 % (1673/2959) in the Renal Insufficiency And Cardiovascular Events (RIACE) study conducted in 2007–2008 [12], 61.6 % (1252/2033) in the Action in Diabetes and Vascular disease: preterAx and diamicroN-MR Controlled Evaluation (ADVANCE) study conducted in 2001–2003 [13], and 71.3 % (290/407) in the Swedish National Diabetes Register conducted in 2002–2007 [14]. These results suggest that normoalbuminuric renal insufficiency is not uncommon among diabetic patients, especially in type 2 diabetes.

Pathogenesis of normoalbuminuric renal insufficiency in diabetes

There are several possible pathogenic mechanisms that may account for the development of normoalbuminuric renal insufficiency in diabetes. One possibility is that renal ischemia due to intrarenal arteriosclerosis may be related to the development of normoalbuminuric renal insufficiency. A negative correlation between GFR and the intrarenal arterial resistance index was found in type 2 diabetes, regardless of albuminuria stage [21, 22]. In addition, carotid intimal medial thickness, carotid stiffness, and silent cerebral infarction were also reported to be associated with impaired kidney function in type 2 diabetes, independent of microalbuminuria [21, 23].

The other possibility is that genetic susceptibility may contribute to the development of normoalbuminuric renal insufficiency. Polymorphisms of the protein kinase C-β gene were reported to be associated with accelerated decline of estimated GFR (eGFR) in type 2 diabetes without overt proteinuria [24].

Although the increasing use of renin–angiotensin system (RAS) blockade may be related to the increasing prevalence of normoalbuminuric renal insufficiency, the RIACE study showed that the use of RAS blockade was

more common in patients with albuminuric renal insufficiency than in those with normoalbuminuric renal insufficiency [12].

Clinical characteristics associated with normoalbuminuric renal insufficiency in diabetes

The reported clinical characteristics of diabetic patients with normoalbuminuric renal insufficiency include a higher proportion of females, a shorter duration of diabetes, lower prevalence of hypertension, smoking, retinopathy, neuropathy, previous cardiovascular disease, and antihypertensive agents including RAS blockade, lower levels of hemoglobin A1c and triglycerides, and higher levels of hemoglobin and high-density lipoprotein cholesterol, as compared to patients with albuminuric renal insufficiency [10, 12, 25]. In addition, compared to patients with normoalbuminuric preserved renal function, those with normoalbuminuric renal insufficiency are older, more frequently females and non-smokers, and have a higher prevalence of hypertension, dyslipidemia, metabolic syndrome, and previous cardiovascular disease, and higher levels of homeostasis model assessment of insulin resistance [10, 26].

Although our study of 260 type 2 diabetic patients with biopsy-proven diabetic nephropathy (96 females, 106 males; age 58.2 ± 11.4 years) included negative proteinuria as well as trace proteinuria in the normoalbuminuria (normal proteinuria) category based on the new classification of chronic kidney disease [3, 4], 154 (59.2 %) of the 260 patients showed low eGFR (<60 ml/min/1.73 m^2), and 15 (9.7 %) of the 154 patients with low eGFR were not associated with albuminuria (proteinuria) [20]. Table 1 presents the baseline clinical and pathological findings of fifteen patients with normoalbuminuria (normal proteinuria) and low eGFR [7 males, 8 females; an average age 62.5 years (range 49–72 years)] at the time of renal biopsy.

The mean eGFR was 46.0 ml/min/1.73 m^2 (range 25.6–57.2 ml/min/1.73 m^2), the mean duration of diabetes was 7.5 years (range 0–21 years), and the mean hemoglobin A1c level (NGSP) was 7.8 % (range 4.5–11.9 %). The prevalence of diabetic retinopathy, hypertension, dyslipidemia, and history of cardiovascular disease were 50.0 % (6/12), 35.7 % (5/14), 27.3 % (3/11), and 14.3 % (2/14), respectively. Our study demonstrated lower prevalence of hematuria and retinopathy, shorter duration of diabetes, lower systolic blood pressure, and higher hemoglobin level in patients with normoalbuminuria (normal proteinuria) and low eGFR as compared to patients with micro/macroalbuminuria (mild/severe proteinuria) and low eGFR. In addition, when compared to patients with normoalbuminuria (normal proteinuria) and preserved eGFR, those with normoalbuminuria (normal proteinuria) and low eGFR were older. Our findings were consistent with previous reports, and provided additional information on the prevalence of hematuria. Furthermore, our study showed that aging was associated with low eGFR regardless of albuminuria (proteinuria) category, and that hematuria, diabetic retinopathy, and low hemoglobin were associated with the progression of albuminuria (proteinuria) regardless of eGFR category [20].

Pathological characteristics associated with normoalbuminuric renal insufficiency in diabetes

There have been few studies regarding the pathological characteristics of diabetic nephropathy with normoalbuminuric renal insufficiency. A study of 8 long-standing type 1 diabetic women with normoalbuminuria and low GFR (75 ± 10 ml/min/1.73 m^2), as measured by creatinine clearance, found that these patients had more advanced glomerular lesions, such as higher volume fraction of mesangium, greater index of arteriolar hyalinosis, and higher percentage of global glomerular sclerosis than 19 normoalbuminuric women with preserved GFR (115 ± 15 ml/min/1.73 m^2) [27]. Similarly, another study indicated that 23 long-standing type 1 diabetic patients with normoalbuminuria and low GFR (<90 ml/min/1.73 m^2), as measured by either iothalamate clearance or creatinine clearance, had more advanced glomerular lesions, such as more increased glomerular basement membrane width and greater fractional volume of glomerulus occupied by mesangium, when compared with 82 normoalbuminuric patients with preserved GFR (≥90 ml/min/1.73 m^2) [28]. In addition, the Cohen diabetic rat, an animal model of type 2 diabetes which shows progressive depression of renal function without proteinuria or hypertension, was reported to show classical diabetic glomerulosclerosis, including mesangial matrix expansion, thickened basement membranes, and increased deposition of type IV collagen [29].

Our study of 260 type 2 diabetic patients also demonstrated that diffuse lesions, nodular lesions, tubulointerstitial lesions, and vascular lesions in patients with normoalbuminuria (normal proteinuria) and low eGFR (<60 ml/min/1.73 m^2) were more advanced compared to those in patients with normoalbuminuria (normal proteinuria) and preserved eGFR (≥60 ml/min/1.73 m^2) in type 2 diabetes [20].

Fioretto et al. [15, 16] proposed a classification system including three major categories based on renal biopsy lesions in type 2 diabetic patients with microalbuminuria and proteinuria—category I with normal or near-normal renal structure (35 % of patients with microalbuminuria

Table 1 Clinicopathological findings at the time of renal biopsy and outcomes in type 2 diabetic patients with normoalbuminuria (normal proteinuria) and low eGFR <60 ml/min/1.73 m^2

Case		Clinical and pathological findings at the time of renal biopsy									Outcomes			
no.	Gender	Age (years)	eGFR (ml/min/1.73 m^2)	Diabetes duration (years)	Hemoglobin A1c (%) (NGSP)	Diabetic retinopathy	Hypertension	Dyslipidemia	History of CVD	Category of renal lesions	Follow-up (years)	Renal events	CVD events	All-cause mortality
1	Male	69	25.6	2	n.d.[a]	n.d.	n.d.	n.d.	n.d.	III	n.d.	n.d.	n.d.	n.d.
2	Female	71	31.7	4	4.5	(−)	(−)	(−)	(−)	II	4.6	(−)	(−)	(−)
3	Male	56	32.1	15	7.1	n.d.	(−)	(−)	(−)	II	16.4	(−)	(−)	(−)
4	Female	61	36.1	1	6.5	(−)	(+)	(−)	(−)	III	5.1	(−)	(−)	(−)
5	Female	64	39.2	21	8.0	(−)	(+)	n.d.	(−)	II	14.5	(+)	(−)	(−)
6	Female	53	41.3	6	7.5	(+)	(+)	(−)	(+)	III	7.0	(−)	(−)	(−)
7	Female	61	49.4	10	6.5	(−)	(−)	(+)	(+)	III	21.8	(−)	(+)	(−)
8	Male	72	51.2	17	11.9	(+)	(−)	(−)	(−)	III	3.6	(−)	(−)	(−)
9	Male	65	52.7	5	5.9	(−)	(−)	(−)	(−)	III	5.3	(−)	(−)	(+)
10	Male	64	53.0	2	7.0	(−)	(−)	(−)	(−)	III	5.6	(−)	(−)	(−)
11	Male	61	53.7	0[b]	6.0	(−)	(−)	n.d.	(−)	III	13.7	(−)	(−)	(−)
12	Female	64	55.5	10	11.8	(+)	(+)	(−)	(−)	III	2.7	(−)	(+)	(−)
13	Female	63	55.7	10	9.9	(+)	(−)	(+)	(−)	II	5.3	(−)	(−)	(−)
14	Female	65	56.0	1	8.0	(+)	(+)	(+)	(−)	II	2.1	(−)	(−)	(−)
15	Male	49	57.2	8	8.3	(+)	(−)	n.d.	(−)	II	13.4	(−)	(+)	(−)

Presence of diabetic retinopathy, hypertension, dyslipidemia, and history of CVD are indicated by (+). Category of renal lesions was evaluated according to the classification proposed by Fioretto et al. [15, 16]

eGFR estimated glomerular filtration rate, *CVD* cardiovascular disease, *n.d.* no data, *NGSP* National Glycohemoglobin Standardization Program

[a] This patient had poorly controlled type 2 diabetes, which was followed at another hospital

[b] This patient was diagnosed for the first time with type 2 diabetes by a random blood glucose test and an oral glucose tolerance test at the time of renal biopsy