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Nodular lesions in diabetic nephropathy: Collagen staining and renal prognosis ☆

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ABSTRACT

Aims: Nodular lesions are one of the most characteristic pathological changes of advanced diabetic nephropathy (DN). Previous studies have demonstrated that the pattern of both routine and collagen staining of nodular lesions changes during their development. However, the association between such changes of staining and the renal prognosis remains unclear.
Methods: Among 252 patients with biopsy-proven DN, 67 met the selection criteria and were enrolled to investigate this relationship. In all patients, nodular lesions were stained with periodic acid Schiff, periodic acid methenamine silver, and Masson trichrome stains, and immunostaining was done for type I, III, IV, V, and VI collagen. The endpoint was commencement of dialysis due to end-stage renal disease.
Results: At least one mesangiolytic nodular lesion (MNL) that showed faint staining for PAS and PAM was found in 61% of the patients. MNLs were negative for type IV collagen staining, unlike the strong positivity of non-MNLs, while type V and VI collagen staining were strongly positive in all nodular lesions. Cox proportional hazards regression analysis revealed that the hazard ratio (HR) for the endpoint was significantly higher in patients with at least one MNL than in patients with no MNLs after adjustment for known promoters of renal progression (HR: 2.94; 95% confidence interval: 1.24–7.07).
Conclusions: MNLs may reflect characteristic differences of collagen production and could be a useful prognostic indicator in patients with nodular lesions. Further investigation of the mechanism underlying these differences of collagen production could contribute to finding new therapeutic targets for DN.

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1. Introduction

Nodular lesions have been known as a pathological hallmark of human diabetic nephropathy (DN) since they were first described in the glomeruli of eight patients with maturity-onset diabetes by Kimmelstiel and Wilson in 1936 [1]. Consequently, the nodular lesion of DN is often called a Kimmelstiel-Wilson lesion. It is characterized by accumulation of homogeneous eosinophilic material within the mesangium, and often appears as a round accentuation of mesangial expansion [2]. These lesions mainly consist of extracellular matrix components, including type IV collagen (which is also found in normal glomeruli), small lipid particles, and cellular debris [3,4]. It has been reported that two processes, which are detachment of endothelial cells from the glomerular basement membrane and mesangiolysis, play an important role in the formation of nodular lesions [3,5]. Mesangiolysis also represents the disintegration of mesangial matrix of nodular lesions. In addition, previous immunohistochemical studies confirmed unique changes of the major collagens in the nodular lesions of patients with DN [3,6].

In clinical practice, nodular lesions are generally seen in patients with advanced DN, and it has been reported that the presence of at least one nodular lesion is associated with a longer duration of diabetes and less favorable clinical parameters [1,7,8]. After the pathological classification of DN was published by the Renal Pathology Society (RPS), an influence of nodular lesions on the clinical outcome was reported [8,9]. However, the definition of nodular lesion was relatively unclear in the RPS classification, and the relationship of the clinical outcome with staining of these lesions for PAS, PAM, and collagens remains unknown.

Therefore, the present study was performed to investigate both conventional and collagen staining of nodular lesions in patients with biopsy-proven DN. In addition, the renal prognosis was compared between two groups classified according to the staining pattern of nodular lesions and the implications of differences in staining were determined.

2. Materials and methods

2.1. Study population

Among 252 patients with diabetes who underwent renal biopsy at our hospital from March 1985 to March 2013 and were confirmed to have pure DN, which was defined as DN without other coexisting renal diseases (except nephrosclerosis) or kidney transplantation, 67 patients were eligible for this study. Eligibility criteria were as follows: (1) $\geq$5 glomeruli in the biopsy specimen, (2) glomerular class III (at least one nodular lesion and $\leq$50% of global glomerulosclerosis) according to the RPS classification, and (3) a baseline estimated glomerular filtration rate (eGFR) $\geq$ 15 mL/min/1.73 m^2 (Fig. 1). Diagnosis of diabetes was based on the criteria of the Japanese Diabetes Society [10]. DN was diagnosed by at least two renal pathologists and/or nephrologists, and the diagnosis was re-evaluated according to the RPS classification [11]. The study protocol was reviewed and approved by the ethics committee of Toranomon Hospital in June 2014. This study was registered with the University Hospital Medical Information Network (UMIN) in June 2015 (UMIN000017871).

2.2. Laboratory parameters and definitions

The GFR was estimated by using the Japanese modification of the Chronic Kidney Disease Epidemiology Collaboration equation [12]. Baseline urinary protein excretion (UP) was measured in a 24-h urine sample. Microalbuminuria was defined as urinary albumin excretion $\geq$ 30 and <300 mg/gCr in at least two of three consecutive urine specimens obtained immediately before and after renal biopsy, while macroalbuminuria was urinary albumin excretion $\geq$ 300 mg/gCr [13] and overt proteinuria was macroalbuminuria or UP exceeding 1 g/day. HbA1c data are presented as National Glycohemoglobin Standardization Program values according to the recommendations of the Japanese Diabetes Society and the International Federation of Clinical Chemistry [14]. Mean arterial pressure (MAP) was calculated as 2/3 diastolic pressure + 1/3 systolic pressure (mmHg). As in our previous study, the average annual values of clinical parameters (such as UP, MAP, HbA1c and hemoglobin) were calculated during follow-up [8]. Treatment with an angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin II type I receptor blocker (ARB) during follow-up was defined as use of such drugs for more than half of the follow-up period.

2.3. Endpoint

The primary endpoint was defined as commencement of dialysis due to end-stage renal disease (ESRD). None of the patients received kidney transplantation.

2.4. Renal biopsy and pathological assessment

As described previously, the indications for renal biopsy were UP > 0.5 g/day or atypical DN, such as nephritic syndrome with short duration of diabetes and renal involvement without diabetic retinopathy and/or with hematuria, so all renal biopsies were performed for clinical reasons and not research [15,16]. Kidney tissue samples were fixed in formalin, and embedded in paraffin for light microscopy and immunohistochemistry. Paraffin sections were stained with PAS, PAM, or Masson trichrome (MT) by standard procedures, and immunohistochemistry with specific primary antibodies. In all patients, biopsy specimens were also subjected to immunofluorescent staining and electron microscopy (EM) for differentiation from other renal disease, such as membranous nephropathy and IgA nephropathy, and to confirm the characteristic findings of DN, e.g., linear homogenous IgG staining of the glomerular basement membrane (GBM) on immunofluorescence without detection of immune complexes by EM. In one patient, all of the glomeruli examined by immunofluorescence showed global sclerosis, but we differentiated DN from other renal diseases based on the results