New Biomarkers for Diagnosis in Patients with Chronic Kidney Disease (CKD)

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In Japan, major causes of end-stage kidney disease (ESKD) are type 2 diabetic nephropathy, IgA nephropathy, and nephrosclerosis. Renal replacement therapies are hemodialysis (HD) and peritoneal dialysis (PD). Since PD patients have shown some complications, such as peritonitis, infections of the exit sites of catheters, and encapsulated peritoneal sclerosis (EPS), it is necessary to determine infections at an early stage of PD. Although a definite diagnosis in patients with chronic kidney disease (CKD) is made through the biopsy of renal tissue and peritoneum, we cannot always perform such diagnostic procedures. Thus, it is necessary to develop non-invasive diagnostic biomarkers prior to biopsies in CKD patients. The objective of this review is to explain the efficacy of new biomarkers in such patients.

Key words: diabetic nephropathy, IgA nephropathy, peritoneal dialysis, chronic kidney disease (CKD)

Introduction

Chronic kidney disease (CKD) is a worldwide public health problem that affects millions of people from all racial and ethnic groups. According to the 2013 annual report by the Japanese Society of Dialysis Therapy (JSDT) 1), the total number of dialysis patients was 314,180, and the leading cause of end stage kidney disease (ESKD) has been diabetes (43.8%), instead of chronic glomerulonephritis (18.8%), since 1998. In Japan, major causes of ESKD are type 2 diabetic nephropathy, chronic glomerulonephritis, especially IgA nephropathy, hypertensive nephrosclerosis, and polycystic kidney disease. The pathogenesis of diabetic nephropathy includes genetic factors and/or environmental factors such as life style impairment and metabolic syndrome. Since the pathogenesis of IgA nephropathy is still obscure, the efforts made by many investigators around the world have gradually clarified various aspects of the pathogenesis and treatment of IgA nephropathy. In Japan, the major renal replacement therapies are hemodialysis (HD) and peritoneal dialysis (PD), but not renal transplantation. Since PD patients have shown some complications, such as peritonitis, infections of the exit sites of catheters, and encapsulated peritoneal sclerosis (EPS), it is necessary to determine infections at an early stage of PD. It is necessary to develop non-invasive diagnostic biomarkers prior to biopsies in patients with CKD.

The objective of this review is to explain the efficacy of new biomarkers in CKD patients, such as type 2 diabetic nephropathy, IgA nephropathy, and peritoneal fibrosis based on clinicopathological findings.

Type 2 diabetic nephropathy

Although renal biopsy is not performed on all patients with diabetes for a definite diagnosis, clinical diagnostic criteria are usually used in Japan (Figure-1, Table-1). The presence of diabetic retinopathy and/or neuropathy are important findings for clinical diagnosis.

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1. New biomarkers using urine and serum samples

MacIsaac et al.\(^2\) reported markers and risk factors for the development and progression of diabetic kidney disease. Emerging risk factors for the progressive loss of renal function include markers of oxidation and inflammation, profibrotic cytokines, uric acid, advanced glycation end products (AGEs), functional and structural markers for vascular dysfunction, renal structural changes, and tubular biomarkers. They reported that the most promising are serum uric acid and soluble tumor necrosis factor (type 1 and type 2) levels, especially in relation to GFR changes\(^2\).

1) Urine sample
   - Albumin

Microalbuminuria is a major clinical sign in the early stage of diabetic nephropathy. Although significant renal histological changes have already appeared even at the stage of microalbuminuria or impaired glucose tolerance (IGT), it is necessary to develop more sensitive markers for detecting early stage renal injury in patients with type 2 diabetic nephropathy. In Japan, patients with diabetic nephropathy are classified into the following five stages. Stage I (normoalbuminuria stage) shows a normal or increased estimated glomerular filtration rate (eGFR) (more than 30 ml/min of eGFR). Stage II (microalbuminuric stage) shows microalbuminuria (30–299 mg/g Cr of urinary albumin excretion, more than 30 ml/min of eGFR). Stage III (macroalbuminuric stage) shows macroalbuminuria (more than 300 mg/g Cr of urinary albumin excretion, more than 30 ml/min of eGFR). Stage IV (renal failure stage) shows a decrease of renal function (less than 30 ml/min of eGFR). Stage V is a dialysis stage.

Horikoshi et al.\(^3\) have developed a highly sensitive and inexpensive method for testing urinary protein levels that is based on a dye-binding method using erythrosin B. A solution containing a buffer agent (pH 2.3) and surfactants, and a solution of erythrosin B are added to a urine sample. After letting the mixture stand at 37°C for 5 min, the dye-bound protein is measured by a spectrophotometer at 546 nm using a Hitachi 779S automated analyzer (Tokyo, Japan). The calibration curve was linear with a human serum albumin concentration in the range of 2.4–200 mg/l. The detection limit, 2.4 mg/l, was superior to conventional dye-binding methods by one order of magnitude and comparable to a turbidimeric immunoassay (TIA). The erythrosin B method showed an excellent correlation and equal sensitivity with TIA. Spot urine samples from 70 patients who showed (−) or (+) in a dip-stick screening test for proteinuria and 79 healthy controls were analyzed. There was an excellent correlation (r = 0.978, n = 149) between the results given by the proposed method and those by the TIA. This sensitive method to measure urinary protein will be useful for the screening of microalbuminuria because proteinuria in early stages of kidney diseases mostly consist of albumin. A high-performance liquid chromatography (HPLC) assay, which we reported as a sensitive method to detect microalbuminuria\(^4\), is also available but costs the same amount as TIA. The erythrosin B method has a 70% cost savings compared with TIA\(^3\).

- MCP–1, IL–8, MMP–9 and Mindin

Tashiro et al.\(^5\) examined the correlation among the levels of urinary monocyte chemoattractant protein–1 (MCP–1) and interleukin–8 (IL–8), hyperglycemia.
and renal injuries in patients with type 2 diabetic nephropathy. High glucose may stimulate MCP-1 and/or IL-8 production and their excretion into the urine, independent of the phases or pathological lesions of this disease. It is indicated that IL-8 increases in an early stage of diabetic nephropathy, and MCP-1 increases in an advanced stage of diabetic nephropathy. It appears that the measurement of urinary MCP-1 and IL-8 may be useful for evaluating the degree of renal injuries in patients with type 2 diabetic nephropathy. We also determined correlations among the levels of urinary matrix metalloproteinase-9 (MMP-9) and type IV collagen, hyperglycemia, urinary protein, and renal injuries in patients with type 2 diabetic nephropathy. Patients with type 2 diabetic nephropathy were divided into two groups: Group 1, patients with normoalbuminuria or microalbuminuria (0–299 mg/g Cr), and Group 2, patients with macroalbuminuria (> 300 mg/g Cr). The mean level of urinary MMP-9 in patients with type 2 diabetic nephropathy increased in accordance with the clinical stage of the disease. The mean level of urinary type IV collagen in Group 2 patients with diabetic nephropathy was significantly higher than that in Group 1 and healthy controls. The levels of urinary type IV collagen in patients with diabetic nephropathy were also increased in accordance with the clinical stage of the disease. It appears that the measurements of urinary MMP-9 and type IV collagen may be useful for evaluating the degree of renal injuries in patients with type 2 diabetic nephropathy, especially in an early stage.

Increasing evidence indicates that the inflammatory and immune response mechanisms may contribute significantly to the development of diabetic nephropathy. Inflammatory biomarkers should be useful in the diagnosis or monitoring of diabetic nephropathy. Mindin (spondin 2) is a member of the mindin-/F-spondin family of secreted extracellular matrix (ECM) proteins. Previous studies showed that mindin is essential for the initiation of innate immune responses and represents a unique pattern-recognition molecule in ECM proteins. Urinary mindin excretion in patients with type 2 diabetes was increased compared with that in healthy controls, reflecting the stage of diabetic nephropathy. It appears that urinary mindin is a potential biomarker in the development of diabetic nephropathy patients.

2) Blood sample
   • TNF receptors

Chronic inflammation promotes the progression of diabetic nephropathy. A number of studies have documented significantly higher circulating concentrations of tumor necrosis factor (TNF) pathway related molecules such as TNFα and TNF receptors (TNFRs) in CKD patients, and those levels are closely correlated with the change of GFR. TNFα, a pleiotrophic cytokine, may play important inflammatory roles in various renal diseases such as lupus nephritis, anti-neutrophil cytoplasmic antibody (ANCA)–associated glomerulonephritis, and renal allograft rejection. However, the role of TNFα remains unclear in patients with type 2 diabetic nephropathy. TNFα is a functional 26kDa homotrimer type II transmembrane protein. It is a central proinflammatory cytokine that is generated in a wide variety of cells, including monocytes, macrophages and T cells, fat cells, and endothelial cells. Gohda et al. reported that the concentrations of TNFRs strongly predict early and late renal function loss in both type 1 and 2 diabetes, independent of classical risk factors such as GFR and albuminuria. Type 1 diabetic patients with normo- or microalbuminuria and with TNFR2 levels in the highest quartile had a 55% cumulative incidence rate of reaching CKD stage 3, compared with a less than 15% incidence rate for patients with TNFR2 levels in the lower 3 quartiles after 12 years of follow-up. Type 2 diabetic patients with proteinuria and with TNFR1 levels in the highest quartile had a nearly 80% cumulative incidence of progression to ESKD after 12 years of follow-up, the rate was less than 20% in those with TNFR1 levels in the lowest 3 quartiles. Furthermore, the concentration of TNFRs also predicted cardiovascular and all-cause mortality, but these effects were smaller than those observed in ESKD.

IgA nephropathy

1. New biomarkers using serum samples
   • Galactose-deficient IgA1 (Gd–IgA1) immune complex

IgA nephropathy is the most common type of primary chronic glomerulonephritis worldwide. IgA nephropathy has a significant morbidity, culmi-
nating in ESKD in about 40% of patients within 20 years of the diagnosis (Figure 2-3). Renal biopsy is required for the diagnosis of IgA nephropathy. We have already reported the importance of four clinical markers in the diagnosis of patients with IgA nephropathy, or in the differential diagnosis from other types of primary chronic glomerulonephritis, as follows: 1) more than five red blood cells (RBCs) in urinary sediments, 2) persistent proteinuria (urinary protein of more than 0.3 g/day), 3) a serum IgA level of more than 315 mg/dL, and 4) a serum IgA/C3 ratio of more than 3.01. Patients with three or four clinical markers were easily diagnosed as having IgA nephropathy in our previous reports. However, it is necessary to develop new biomarkers for the early detection of
this disease without renal biopsy. Several recent studies suggest that aberrant O-glycosylation of circulatory IgA1 is vital in the pathogenesis of IgA nephropathy. The O-linked glycans in the hinge region of IgA1 are generally composed of N-acetylgalactosamine (GalNAc) and galactose; sialic acid may be attached to either or both sugars. IgA1-producing cells secrete a mixture of IgA1 O-glycoforms. Studies in the different populations have shown that IgA nephropathy patients have significantly higher levels of circulating IgA1 with galactose-deficient, O-linked, hinge-region glycans. Depending on the population studied, 50-75% of IgA nephropathy patients have levels above the 90th percentile for healthy controls. In addition, IgA1 eluted from renal tissues of IgA nephropathy patients also exhibits a galactose deficiency in the O-linked glycans in the hinge-region.

The serum level of IgA1-containing circulating immune complexes is elevated in patients with IgA nephropathy. These complexes contain galactose-deficient IgA1 (Gd-IgA1) bound by IgG and/or IgA antibodies. Recently, we have shown that the IgG auto-antibodies that recognize glycan-containing epitopes on Gd-IgA1 exhibit unique features in the complementarity-determining region 3 of the variable region of their heavy chains. Furthermore, the serum levels of IgG auto-antibodies specific for Gd-IgA1 correlated with disease severity, as assessed by the magnitude of proteinuria. However, the serum levels of Gd-IgA1-containing circulating immune complexes may differ widely among IgA nephropathy patients. Furthermore, some IgA nephropathy patients do not show glomerular deposition of IgG, but rather only IgA. Therefore, it is difficult to explain the pathogenesis of IgA nephropathy by an elevated serum level of glycan-specific antibodies of only the IgG isotype. These latter features may be explained by our observation that some patients with IgA nephropathy have complexes generated by glycan-specific antibodies of the IgA1 isotype. Whereas the serum levels of IgA, Gd-IgA1, and glycan-specific IgG are higher in patients with IgA nephropathy compared with healthy controls, the levels of these parameters have not been systematically studied in patients with other kidney diseases with clinical features similar to those of IgA nephropathy. Therefore, we examined the prevalence of elevated serum levels of IgA, Gd-IgA1, and glycan-specific IgG and IgA in IgA nephropathy patients and a large cohort of CKD patients to assess the utility of these biomarkers for the non-invasive diagnosis of IgA nephropathy. It was revealed that this panel of biomarkers is helpful in differentiating patients with IgA nephropathy from patients with other glomerular diseases. Yanagawa and Suzuki et al. compared the serum levels of IgA, IgG, Gd-IgA1, Gd-IgA1-specific IgG, and Gd-IgA1-specific IgA in 135 IgA nephropathy patients, 79 patients with non-IgA nephropathy CKD, and 106 healthy controls. Serum was collected at the time of renal biopsy from all IgA nephropathy and non-IgA nephropathy CKD patients. Each serum marker was significantly elevated in IgA nephropathy patients compared with non-IgA nephropathy CKD patients (p<0.001) and healthy controls (p<0.001). While 41% of IgA nephropathy patients had elevated serum Gd-IgA1 levels, 91% of these patients exhibited Gd-IgA1-specific IgG levels above the 90th percentile for healthy controls (sensitivity 89%, specificity 92%). Although up to 25% of non-IgA nephropathy CKD patients, particularly those with immune-mediated glomerular diseases including lupus nephritis, also had an elevated serum levels of Gd-IgA1-specific IgG, most IgA nephropathy patients had elevated levels of Gd-IgA1-specific antibodies of both isotypes. Serum levels of Gd-IgA1-specific IgG were associated with renal histological grading. Furthermore, there was a trend toward higher serum levels of Gd-IgA1-specific IgG in IgA nephropathy patients with at least moderate proteinuria (more than 1.0 g/g Cr), compared with the patients with less proteinuria. In conclusion, serum levels of Gd-IgA1-specific antibodies are elevated in most IgA nephropathy patients, and their assessment, together with serum levels of Gd-IgA1, improves the specificity of the assays. It appears that a panel of serum biomarkers may be helpful in differentiating IgA nephropathy from other glomerular diseases.

Peritoneal injury in peritoneal dialysis (PD)

1. New biomarkers using peritoneal fluid
   - Pentraxin 3 (PTX3)

PD is one effective treatment for ESKD patients.
However, it is well known that peritonitis and/or bioincompatible peritoneal dialysate may play an important role in the development of peritoneal fibrosis. These pathological alterations of the peritoneal membrane in long-term PD patients may progress to EPS, i.e. a serious complication in PD patients (Figure-4). Changes of peritoneal solute transport in long-term PD patients often results from an increased vascular surface area with vasculopathy. Angiogenesis and vasculopathy in the peritoneum may play an important role in the regulation of water and solute transportation in the peritoneum. However, the relationship between vascular changes and the development of peritoneal fibrosis is still obscure. Simple sclerosis consists of a thin layer of submesothelial sclerotic tissues in PD patients. EPS was characterized by the thickening sclerotic tissues involving vascular alterations in PD patients.

It is well-known that biocompatible peritoneal dialysate may play a central role in the development of peritoneal fibrosis. Peritoneal inflammation continues even after the cessation of peritoneal dialysate stimulation. It is important to establish a definition of persistent inflammation in the peritoneal cavity at the cessation of PD. Pentraxin 3 (PTX3) is characterized as a member of the long pentraxin superfamily. It shares the C-terminal pentraxin domain with short pentraxins such as CRP and serum amyloid P components, but differs in terms of the presence of an unrelated long N-terminal domain, cellular source, and regulatory mechanism. PTX3 is synthesized locally at the inflammatory site from various kinds of cells, including endothelial cells, smooth muscular cells, and fibroblasts. Kanda and Hamada et al. determined whether PTX3 in the peritoneal effluent (PE) may be a new biomarker in PD patients. PTX3 in serum, PE, and peritoneal specimens were obtained from 50 patients with ESKD in our hospital. Samples of 19 patients were obtained at the initiation of PD and those of 31 patients at the cessation of PD. PTX3, high-sensitivity CRP, matrix metalloproteinase-2 (MMP-2), and IL-6 were analyzed. An immunohistological examination using an anti-PTX3 antiserum was also performed. Expressions of PTX3 were observed in the endothelial cells, fibroblasts, and mesothelial cells in the peritoneum. The PTX3 level in PE at the cessation of PD was significantly higher than that at the initiation of PD. Effluent PTX3 levels in patients with a history of peritonitis or a PD duration of more than 8 years were significantly higher than those in patients without peritonitis or in patients with a PD duration of less than 8 years. The PTX3 level was significantly correlated with MMP-2 and IL-6 levels in the PE, as well as the thickness of the submesothelial compact (SMC) zone and the vasculopathy. It appears that PTX3 may be a new biomarker of peritoneal inflammation and progressive fibrosis.

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