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Clinical and Pathological Significance of Autoantibodies to Erythropoietin Receptor in Type 2 Diabetic Patients With Chronic Kidney Disease

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Introduction: We examined the impact of autoantibodies on the erythropoietin receptor (EPOR) in type 2 diabetic patients with chronic kidney disease (CKD).

Methods: A total of 112 Japanese patients with type 2 diabetes who had CKD were enrolled in this study and followed for a mean of 45 months. Sera from these patients were screened for anti-EPOR antibodies using enzyme-linked immunosorbent assays.

Results: Anti-EPOR antibodies were detected in 26 patients (23%). Anti-EPOR antibodies were associated with low hemoglobin concentrations and decreased renal function. In patients with biopsy-proven diabetic nephropathy, anti-EPOR antibodies were associated with increased levels of interstitial inflammation. A decrease in renal function was observed more frequently in patients with antibodies than in those without antibodies, and the presence of the antibodies together with well-known clinical parameters, including proteinuria and low glomerular filtration rate, was a significant risk factor for end-stage renal disease. In human tubular epithelial HK-2 cells, IgG fractions containing anti-EPOR antibodies upregulated the expression of monocyte chemoattractant protein-1 mRNA under a high concentration of glucose.

Discussion: Anti-EPOR antibodies might be involved in the progression of renal lesions and in the impaired erythropoiesis in type 2 diabetic patients with CKD. Furthermore, the presence of anti-EPOR antibodies may be an additional predictor for end-stage renal disease in type 2 diabetes.

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Diabetes and its complications are major causes of morbidity and mortality in most countries.[1] Among diabetic complications, nephropathy occurs in 20% to 40% of patients during the course of their disease.[2] Although kidney disease attributable to diabetes is referred to as diabetic nephropathy or diabetic kidney disease, diabetes and various kidney diseases have become common chronic conditions.[3] Thus, the prevalence of chronic kidney disease (CKD) is increasing in proportion to the increase in diabetes, and it has been predicted to continue to increase in the future.[4] Diabetes is a risk factor for cardiovascular disease and death, and CKD increases this risk further.[5–8] Anemia is commonly observed in diabetic patients with CKD and is involved in the increased risk for the progression of kidney disease and cardiovascular mortality and morbidity.[7,9–11]

Anemia in diabetic patients with CKD may result from ≥1 mechanisms. Among these, the major causes are iron and erythropoietin (EPO) deficiencies, as well as hyporesponsiveness to the action of EPO.[11] In view

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of EPO hyporesponsive anemia, we previously detected and reported autoantibodies to the EPO receptor (EPOR) as a possible cause of anemia with erythroid hypoplasia.[12] In that study, these antibodies were unexpectedly detected even in diabetic patients with CKD, although their clinical significance remains to be investigated.[12] In addition, a recent study revealed that anti-EPOR antibodies were associated with overall disease activity and the decline of renal function in patients with systemic lupus erythematosus.[13]

These results prompted us to examine the clinical and pathological impact of anti-EPOR antibodies in type 2 diabetic patients with CKD. We found that anti-EPOR antibodies were detected in a subset of patients, were associated with renal lesions, and that they were inversely related to the preservation of renal function.

MATERIALS AND METHODS

Patients

A total of 112 type 2 diabetic patients who had been diagnosed with CKD and followed at Kanazawa University Hospital between 1989 and 2014 were included in this study. The mean follow-up was 45.3 ± 47.4 months. Patients with secondary diabetes, renal transplantation, or dialysis were excluded. Among the enrolled patients, 51 underwent a renal biopsy. A diagnosis of diabetic nephropathy was confirmed by histological characteristics using renal biopsy specimens, including light microscopy, electron microscopy, and immunofluorescence examination. A renal biopsy was performed for the precise diagnosis of renal lesions with the consent of each patient. All blood samples were obtained after the patients gave their written informed consent at admission for the renal biopsy, or for workup and treatment of the disease. The study protocol adhered to the Declaration of Helsinki and was approved by the medical ethics committee of Kanazawa University. In addition to 40 previously reported healthy control subjects,[12] a further 8 healthy individuals were included as control subjects in the present study, and as a result, anti-EPOR antibodies were not detected in serum samples from any of these subjects.

Clinical Features and Routine Laboratory Tests

Demographic and clinical features were evaluated for each enrolled patient. Baseline clinical and laboratory findings, including the use of angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and erythropoiesis-stimulating agents, were extracted from medical records. Twenty-four hour urinary protein excretion, serum creatinine, estimated glomerular filtration rate (eGFR), glycosylated hemoglobin (HbA_{1c}), body mass index, systolic and diastolic blood pressures, total cholesterol, hemoglobin (Hb), iron, total iron binding capacity, ferritin, and C-reactive protein were used as baseline clinical parameters at admission. eGFR for Japanese patients was calculated using the following equation: eGFR (ml/min per 1.73 m^2) = 194 × serum creatinine$^{-1.094}$ × age$^{-0.287}$ (if female, × 0.739).[14] HbA_{1c} levels were presented as National Glycohemoglobin Standardization Program values according to the recommendations of the Japan Diabetic Society[15] and the International Federation of Clinical Chemistry values. Autoantibodies against glutamic acid decarboxylase were measured by a commercial quantitative enzyme-linked immunosorbent assay (SRL, Inc., Tokyo, Japan).

Detection of Autoantibodies to EPOR

Anti-EPOR antibodies were detected by enzyme-linked immunosorbent assay as described previously.[12] Briefly, polyvinyl 96-well microtitration plates (Nunc International, Tokyo, Japan) were coated with recombinant human EPOR (R & D Systems, Minneapolis, Minnesota) at 5 μg/ml diluted in 0.2 M sodium bicarbonate buffer at 4°C for 24 hours. The remaining free-binding sites were blocked with 1% bovine serum albumin in phosphate-buffered saline at 4°C. After the plates were washed with Tween 20-Tris-buffered saline, the samples were added in duplicate at 1:1000 dilution to 1% bovine serum albumin in phosphate-buffered saline for 20 hours at 4°C. The plates were washed 4 times with the same buffer and incubated with goat antihuman Ig-conjugated with horseradish peroxidase (Millipore, Temecula, California) at 1:5000 dilution for 1.5 hours at room temperature. The substrate tetramethylbenzidine (KPL, Gaithersburg, Maryland) was added, and the reaction was stopped by the addition of 2 N sulfuric acid. The optical density at 450 nm (OD_{450}) was determined by an automatic plate reader, and the sample was considered to be antibody-positive when the ratio of patient serum OD_{450} to that of normal control sera was ≥2.4. This cutoff OD value was determined by a preliminary analysis using a receiver-operating characteristic curve to predict, sensitively and specifically, renal outcome among the enrolled type 2 diabetic patients with a positive OD value ≥1.5 (data not shown).[12]

Purification of IgG Fractions

Sera from patients and control subjects were clarified by centrifugation at 1500*g* for 20 min (500 μl) and filtration through 0.45-μm filters (Millipore, County Cork, Ireland). IgG fractions were prepared using a MAb Trap Kit (GE Healthcare, Tokyo, Japan) according