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Clinical predictive biomarkers for normoalbuminuric diabetic kidney disease

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ABSTRACT

Aims: A portion of patients with diabetes mellitus follow the progression of a non-albuminuria-based pathway; i.e., normoalbuminuric diabetic kidney disease (NA-DKD). However, the risk factors which determine NA-DKD are not yet fully understood. This cross-sectional study was therefore aimed to investigate the association between various biomarker levels and estimated glomerular filtration rate (eGFR) in patients with type 2 diabetes mellitus and normoalbuminuria (T2D-NA).

Methods: We measured cardiovascular disease (CVD) [serum osteoprotegerin (OPG), plasma brain natriuretic peptide (BNP), cardio-ankle vascular index (CAVI)], tubular damage [urinary L-type fatty acid binding protein (L-FABP)], and inflammatory [serum tumor necrosis factor (TNF) α and its receptors (TNFRs)] biomarkers in 314 patients with T2D-NA.

Results: The biomarkers of CVD and inflammation showed a significant negative correlation with eGFR. In a logistic multivariate model, none of the biomarkers, except TNFα and TNFRs, were associated with reduced renal function (eGFR < 60 mL/min/1.73 m^2) after adjustment for possible biological and clinical covariates. However, the association observed in TNFα was lost after adjusting for TNFR and other covariates.

Conclusions: In patients with T2D-NA, elevated levels of circulating TNFRs, but not of TNFα, were strongly associated with reduced renal function, independently of all relevant covariates.

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1. Introduction

The development of microalbuminuria has been considered to be an initial clinical sign of diabetic nephropathy (DN), which leads to macroalbuminuria and then to progressive decline of glomerular filtration rate (GFR) and eventually end-stage renal disease. However, the natural course of DN has been recently challenged, as approximately 10–30% of patients with both type 1 and type 2 diabetes mellitus start renal function decline before the onset of microalbuminuria or macroalbuminuria [1,2]. Previous studies found that patients with normoalbuminuric diabetic kidney disease (NA-DKD) were predominantly female, older in age, and had a lower prevalence of retinopathy, among other factors [3,4]. Moreover, chronic low-grade inflammation, atherosclerosis, and tubulointerstitial or vascular damage have been shown to play key roles in the pathophysiology of NA-DKD [3,5]. However, the specific biomarkers that determine NA-DKD are not yet fully understood.

In the present study, we measured several types of biomarkers for cardiovascular disease (CVD) [serum osteoprotegerin (OPG), plasma brain natriuretic peptide (BNP), cardio-ankle vascular index (CAVI)], tubular damage [urinary L-type fatty acid-binding protein (L-FABP)], and inflammation [serum tumor necrosis factor (TNF) α and its receptors (TNFRs)], and investigated the association between various biomarker levels and estimated GFR (eGFR) in patients with type 2 diabetes mellitus and normoalbuminuria (T2D-NA).

2. Subjects, materials and methods

2.1. Study design

Diabetes mellitus was diagnosed based on the diagnostic criteria of the Japan Diabetes Society [6]. Overall, 738 patients with diabetes mellitus were recruited for observation of the natural course of DKD at Kure Medical Center and Chugoku Cancer Center. The enrollment spanned a period between July 1, 2014, and March 31, 2016. We selected only patients with diabetes mellitus type 2 and excluded 99 participants with type 1 diabetes (n = 80) or other types of diabetes (n = 19). We also excluded participants with micro- (n = 186) and macroalbuminuria (n = 113). Additionally, 17 participants did not undergo checkups for fundoscopy, and nine participants had missing information for some values [CAVI (n = 4), L-FABP (n = 2), BNP (n = 3)]. In the end, we included data from 314 participants with T2D-NA. The algorithm for the selected participants is shown in Fig. 1. This study was approved by the ethics committee of Kure Medical Center and Chugoku Cancer Center. Informed consent was obtained from all patients, and this study complies with the Declaration of Helsinki.

Each patient's baseline anthropometric and clinical characteristics were recorded. Body mass index was calculated at weight/height squared (kg/m^2), while eGFR was calculated using the following equation for the Japanese population: eGFR (mL/min/1.73 m^2) = 194 × [age (years)]$^{-0.287}$ × [serum creatinine (mg/dL)]$^{-1.904}$ × 0.739 (for females) [7]. Ophthalmologists used the Davis classification to diagnose diabetic retinopathy as simple, pre-proliferative, proliferative, or not present. Prior CVD was defined as a medical history of coronary or peripheral artery revascularization, myocardial infarction, or stroke. Serum samples were obtained at baseline and stored at −80 °C until use.

2.2. Laboratory measurements

We used enzyme-linked immunosorbent assay to measure serum TNFα (cat. No. KAC1751; Invitrogen, Carlsbad, CA, USA), serum TNFR1 and serum TNFR2 (cat. Nos. DRT100, DRT200; R&D Systems, Minneapolis, MN, USA), and serum OPG (cat. No. BI-20403; Biomedica, Vienna, Austria), as described previously [8]. Plasma BNP (Architect BNP-JP®, Abbot Japan Co., Ltd., Tokyo, Japan), urinary L-FABP (Renischem® L-FABP ELISA high sensitivity kit, CIMIC Holdings Co., Ltd., Tokyo, Japan), and albuminuria were measured using routine laboratory methods. Non-high-density lipoprotein cholesterol (non-HDL-C) was defined as the difference between total cholesterol and HDL-C. Urinary albumin and Cr were quantified using a immunonephelometry (N-assay TIA Micro Alb; Nittobo Medical Co., Ltd., Fukushima, Japan) and enzymatic method, respectively. The urinary albumin to creatinine ratio (ACR) was expressed as milligrams per gram of Cr. CAVI was measured using the VaSera VS-1500A vascular screening system (Fukuda Denshi Co. Ltd., Tokyo, Japan) with the patients resting in a supine position.

2.3. Statistical analyses

All variables are expressed as percentages for categorical data and as mean (standard deviation; SD) or median and interquartile range. The distribution of biomarker concentrations was right-skewed, and we handled biomarkers as continuous variables after common logarithmic transformation. We used these log values for the analysis of differences between the groups and Pearson correlation analysis among different biomarkers. We then further standardized the logarithmic transformed biomarker values for the multivariate analysis to facilitate comparisons using the following formula: (logarithmic transformed biomarker value – average value of logarithmic transformed biomarker)/SD value of logarithmic transformed biomarker. For analytical purposes, patients were stratified according to their eGFR [NA-DKD: eGFR < 60; vs. no chronic kidney disease (No-CKD): eGFR ≥ 60 (mL/min/1.73 m^2)]. Differences between groups were tested with Mann-Whitney *U* test. Pearson correlation analysis was used to assess the associations among clinical parameters and biomarkers. Using a multivariate logistic regression model, we evaluated association of various biomarkers with the presence of NA-DKD. Candidate covariates were chosen as follows: age and sex were included into adjusted models based on biological plausibility; then, we considered inclusion of covariates associated with the presence of NA-DKD based on findings from our univariate logistic regression analyses.