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Decreased plasma α-Klotho predict progression of nephropathy with type 2 diabetic patients

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ABSTRACT

Aim: The potential role of soluble α-klotho in diabetic kidney disease has not yet been evaluated. The aim of this study was to evaluate the association of plasma and/or urine α-klotho with the progression of type 2 diabetic nephropathy.
Methods: The baseline values of plasma and urine α-klotho were measured in 147 patients with type 2 diabetes mellitus with an estimated glomerular filtration rate (eGFR) of ≥60 mL/min/1.73 m^2. In this prospective observational study, a total of 109 type 2 diabetic patients were followed up for 34 months (8–50 months).
Results: Plasma α-klotho, but not urine α-klotho, was negatively correlated with the decline of eGFR ($r = -0.304$, $P = 0.001$; $r = 0.042$, $P = 0.068$, respectively). After adjusting for several clinical parameters, baseline eGFR and urine ACR, plasma α-klotho was significantly associated with the decline of eGFR ($r = -0.219$, $P = 0.008$). In the normoalbuminuria group (n = 63), the plasma α-klotho remained significantly associated with a decline in eGFR ($r = 0.324$, $P = 0.004$) in the final model.
Conclusions: It is suggested that plasma α-klotho may be an early biomarker for predicting renal impairment in type 2 diabetic patients. The disappearance of a compensatory increase of plasma α-klotho might be a predictive marker for the progression of type 2 diabetic nephropathy.

1. Introduction

Diabetic nephropathy occurs in 20–40% of all patients with type 2 diabetes mellitus (Ahn et al., 2014; American Diabetes Association, 2015). It is a microvascular complication with high morbidity and mortality, as well as being the leading cause of end-stage renal disease (ESRD) (Collins et al., 2012). Although albuminuria and decreased glomerular filtration rate (GFR) are considered to be the main clinical markers for the development and progression of diabetic nephropathy, significant renal damage has already occurred by the time these signals are present (Barratt & Topham, 2007).

The Klotho gene was identified as an aging suppressor gene that extends life span when overexpressed and accelerates aging when disrupted (Akimoto et al., 2012). It is expressed as a transmembrane protein in predominantly the distal convoluted tubules in the kidney and choroid plexus in the brain (Kuro-o et al., 1997). It is also expressed in several endocrine organs such as the pituitary, pancreas, parathyroid gland, adipocyte and vascular endothelial cells (Ben-Dov et al., 2007; Chihara et al., 2006; Donate-Correa et al., 2013; Kuro-o et al., 1997). Unlike membrane Klotho, secreted Klotho is known to regulate the activity of multiple glycoproteins on cell surfaces, including ion channels and growth factor receptors such as insulin/insulin-like growth factor-1

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receptors (Kuro-o, 2010). Several studies have reported that patients with chronic kidney disease have reduced plasma and urine α-klotho levels in the early stages of kidney disease, progressively decreasing in more advanced stages (Akimoto et al., 2012; Hu et al., 2011; Kim et al., 2013).

The role of α-klotho in the pathogenesis of diabetic nephropathy has not been fully elucidated. Recently, we demonstrated that the plasma and urine soluble α-klotho levels were significantly increased in the diabetic patients with relatively preserved renal function (eGFR ≥60 mL/min/1.73 m^2) compared to healthy control subjects, and plasma α-klotho levels decreased in proportion to urinary albumin excretion, although urine α-klotho levels were stable with increasing urinary albumin excretion in our cross-sectional study (Lee et al., 2014). However, it is unclear whether the changes in plasma and urine α-klotho precede the development of albuminuria and decline of GFR in the early development and progression of diabetic nephropathy.

The aim of this study was to evaluate the impact of plasma and urine α-klotho on the early development and progression of type 2 diabetic nephropathy and to determine whether plasma and urine α-klotho are associated with the decline in eGFR and development and/or progression of albuminuria in type 2 diabetic patients with eGFR ≥60 mL/min/1.73 m^2.

1.1. Study Subjects

This study analyzed subjects enrolled in a prospective observational study of early biomarkers for diabetic nephropathy (Diabetic Kidney Disease Study [DKDS]) at Pusan National University Hospital in Busan, Korea (Kim et al., 2014; Lee et al., 2014). This study was carried out in accordance with the Declaration of Helsinki and the protocol was approved by the Institutional Review Board of Pusan National University Hospital (20100024). All patients provided written informed consent before enrollment. Data are available to all interested researchers on request to the Institutional Review Board of Pusan National University Hospital.

A total of 147 Korean type 2 diabetic patients were enrolled consecutively at outpatient clinics between February 2010 and February 2012. The inclusion and exclusion criteria for eligibility have been described (Kim et al., 2014). Briefly, all enrolled patients had relatively conserved renal function (estimated glomerular filtration rate [eGFR] ≥60 mL/min/1.72 m^2, and serum creatinine <1.2 mg/dL). Estimated GFR of 60 is considered as the threshold value of eGFR for the current definition of chronic kidney disease (CKD) (Levey et al., 2011). In addition patients had a sufficient washout period for RAS (renin–angiotensin system) inhibitors (no history of administration of RAS inhibitors at enrollment or a washout period for these drugs of at least 2 months before enrollment). Patients who had disorders/status that affect renal function or urinary samples were excluded (active urinary tract infection; renal disease other than diabetic nephropathy; neoplastic disorders; severe liver dysfunction; active or chronic infection or inflammatory disorders; pregnancy; or a recent [within 6 months] history of acute myocardial infarction, stroke, or occlusive peripheral vascular disease).

Random spot urine and blood samples were obtained from each patient at their clinic visit. Medical histories and anthropometric measurements were also recorded at the same visit. The patients were followed up at our clinic until September 2014. Of these 147 patients, 38 were excluded during follow-up for the following reasons: 19 patients were lost during follow-up (withdrawal of study, n = 10; data not available, n = 9); 3 patients had a follow-up of relatively short duration (<1 year); 10 patients were hospitalized for other severe acute and chronic diseases (acute myocardial infarction, acute stroke, pneumonia, rheumatoid arthritis, hemoptysis, cholangitis and iatrogenic Cushing syndrome); 5 patients were diagnosed with additional malignancies, and 1 patient died of other causes during the follow-up period. Finally, a total of 109 were enrolled in this study. Urine and blood samples were taken at intervals of 12 ± 1 (mean ± SD) months at the outpatient clinic during their follow-up period. Serum creatinine (for calculation of eGFR) and urine albumin-to-creatinine ratio (ACR) were measured at intervals of 6 ± 1 (mean ± SD) months during the follow-up period using the same method.

1.2. Measurements

The eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula: MDRD = 186 × (serum creatinine [mg/dL])$^{-1.154}$ × (age in years)$^{-0.203}$ (Myers et al., 2006). A correction factor of 0.742 was used for females. The annual decline in eGFR was calculated by dividing the change in eGFR by follow-up duration. Urine ACR was measured from spot urine. Albuminuria values were defined as follows: normoalbuminuria (ACR, <30 mg/g creatinine), microalbuminuria (ACR, 30–299 mg/g creatinine) and albuminuria (ACR, ≥300 mg/g creatinine). Plasma and urine concentrations of soluble α-klotho were analyzed using a commercial ELISA kit, as described (Lee et al., 2014). Briefly, plasma samples were centrifuged for 15 min at 3,000 rpm within 30 min of collection; plasma was removed and stored at −70 °C until analysis. Urine samples were centrifuged for 10 min at 3,000 rpm to remove particulate matter and stored at −70 °C until analysis. The plasma and urine concentrations of α-klotho were analyzed using human soluble α-klotho immunoassay kits (Immuno-Biological Laboratories, Gunma, Japan) according to the manufacturer's protocol. Samples were analyzed in duplicate and were within the range of the standard curve (93.75–6,000 pg/mL); values below the detection limit (6.15 pg/mL) were approximated using the mean value between zero and the lower limit of detection. The intra- and inter-assay coefficients of variation were less than 10%. The data on urinary α-klotho were expressed as ratios of urinary α-klotho to urinary creatinine (urine α-klotho/Cr) to assess the hydration states and renal functions of the patients.

1.3. Statistical analysis

Statistical analyses were performed using SPSS version 15.0 (SPSS, Chicago, IL, USA). We divided the diabetic patients into tertile groups according to their baseline plasma and urine α-klotho levels, respectively. Data were presented as means ± SD for normally distributed values and medians (interquartile range) for nonparametric values. Distributions of continuous variables were examined for skewness and kurtosis, and logarithm-transformed values of variables with nongaussian distribution were used for analyses. Differences between groups were analyzed by ANOVA, followed by Bonferroni's test for normally distributed values or the Kruskal–Wallis test for nonparametric values. Categorical variables were reported as frequencies and proportions. Pearson's χ^2 test was employed to analyze categorical data as appropriate. Pearson correlation coefficient was used to test the correlations between individual continuous variables. We conducted multivariate regression analyses with annual rates of decline in eGFR as dependent variables and plasma and urine α-klotho as independent variables. Several models were constructed to adjust for confounding factors including age, sex, HbA1c, systolic BP, HDL cholesterol, duration of diabetes, baseline eGFR and ACR. A multivariate analysis for albuminuria persistence or progression, using an enter procedure was conducted including factors with a p value of <0.2 in the univariate analysis. The multivariate Cox regression model for microalbuminuria/albuminuria persistence or progression was adjusted for age, HbA1c, SBP, HDL cholesterol, baseline eGFR and ACR. A p value of <0.05 derived from the two-tailed Student's t-test was considered to indicate statistical significance.

2. Results

2.1. Baseline clinical characteristics

Patients were divided into tertile groups according to their plasma and urine α-klotho (based on Urine α-klotho/Cr) levels, respectively