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Intact fibroblast growth factor 23 levels predict incident cardiovascular event before but not after the start of dialysis

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ABSTRACT

Purpose: Low 25-hydroxyvitamin D (25D), increased levels of fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH), and alkaline phosphatase (ALP) were reported to be risk factors for mortality in chronic kidney disease (CKD). However, the independent associations of these factors with cardiovascular disease (CVD), the leading cause of death among CKD patients, remain unclear. Our purpose was to identify which of these factors predict incident CVD in CKD.

Methods: In this prospective cohort study, we enrolled 738 predialysis outpatients in the two nephrology departments. We employed Cox proportional hazards analyses to elucidate predictors of the endpoint, defined as fatal or non-fatal cardiovascular event requiring hospitalization. Multiple imputation was performed for missing values.

Results: Mean estimated glomerular filtration rate (eGFR) was 35 mL/min/1.73 m^2. During a median duration of 4.4 years, 86 patients developed the endpoint, of whom 62 patients achieved it before the initiation of dialysis. Multivariable analyses revealed that high serum intact FGF23 levels predicted the outcome preceding dialysis initiation (hazard ratio (HR) per lnFGF23 (SD), 1.64 (1.27–2.30)), while 25D, PTH, and bone-specific ALP did not. Adding FGF23 to the conventional model of age, sex, diabetes, prior CVD, pulse pressure, and eGFR, led to a net reclassification improvement of 6.87% ($P = 0.04$). Not censoring the patients at the start of dialysis and continuing follow-up even after dialysis, FGF23 levels did not predict the outcome (HR, 1.16 (0.91–1.48)). Complete case analyses yielded similar results.

Conclusions: Intact FGF23 levels in predialysis CKD predicted incident cardiovascular events requiring hospitalization before starting dialysis, but did not predict events during the entire follow-up period, including post dialysis initiation.

Introduction

CKD-MBD is an umbrella concept, originally established in dialysis patients, which covers laboratory abnormalities, bone abnormalities and ectopic calcifications [1]. In dialysis patients, studies have revealed that laboratory abnormalities in CKD-MBD are risk factors for incidence of CVD [2,3]. However, data are still scarce among CKD patients not yet on dialysis. Candidates of laboratory abnormality as a risk factor include low concentrations of 25D and high levels of FGF23, PTH, and ALP or BSAP, in addition to hyperphosphatemia [4,5].

Low circulating 25D [6-8] and high PTH [9] levels were reported to be related to mortality in CKD patients. Previous studies showed that approximately half of the deaths in CKD patients were from cardiovascular causes [10,11]. In this regard, these laboratory abnormalities

Abbreviations: CKD-MBD, chronic kidney disease-mineral and bone disorders; CVD, cardiovascular disease; 25D, 25-hydroxyvitamin D; FGF23, fibroblast growth factor 23; PTH, parathyroid hormone; ALP, alkaline phosphatase; BSAP, bone-specific alkaline phosphatase; DM, diabetes mellitus; CAD, coronary artery disease; HF, heart failure; PAD, peripheral arterial disease; BP, blood pressure; Alb, albumin; Ca, calcium; eGFR, estimated glomerular filtration rate; 1,25D, 1,25-dihydroxyvitamin D; SD, standard deviation; IQR, interquartile range; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; MI, multiple imputation; NRI, net reclassification improvement; PP, pulse pressure; BMI, body mass index, KDOQI, National Kidney Foundation Kidney Disease Outcomes Quality Initiative; HR, hazard ratio; 95% CI, 95% confidence interval; KDIGO, Kidney Disease: Improving Global Outcomes.

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might be associated with cardiovascular events in CKD patients. With regard to PTH, the association with prevalent CVD was reported [12], but not for the association with incident CVD.

ALP is an enzyme that catalyzes the hydrolysis of pyrophosphate which has inhibitory effects on vascular calcification [13]. In fact, high ALP levels was reported to be associated with severe coronary artery calcification in dialysis patients, [14] and also reported to be a risk factor for mortality in both hemodialysis [15] and predialysis patients [16]. Among CKD patients without liver disease, ALP levels increase due to high turnover bone disease. Recently, it was found in dialysis patients [17] that the effect sizes related to cardiovascular and non-cardiovascular mortalities were much higher for BSAP in comparison with ALP. However, little data exists in CKD patients not on dialysis.

FGF23 is a bone-derived hormone that maintains phosphate homeostasis and vitamin D metabolism [18] and increases as renal function declines [19,20]. Among patients with CKD, elevated FGF23 levels were shown to be associated with left ventricular hypertrophy [21] and endothelial dysfunction [22], which are known to be risk factors for cardiovascular events and deaths [23-25]. These results suggest a significant association between FGF23 and CVD in CKD. This association was recently reported by a small study [26], in which it did not adjust for important confounder PTH. Therefore, studies of the association between FGF23 and future CVD are still scarce and insufficient in those with CKD not on dialysis.

Observational studies among pre-dialysis CKD patients have constituted insufficient proof of the association of laboratory abnormalities with incident CVD and no study has measured all of these MBD-biomarkers in one study, to our knowledge. In the present study, we measured serum 25D, FGF23, PTH, and BSAP at the same time. The aims of this study were (1) to clarify which of the four markers predict incident cardiovascular events in patients with CKD and (2) to assess the extent to which the addition of significant MBD-biomarkers to the conventional model improves the accuracy of prediction.

Materials and methods

Study population

The study population consisted of 738 predialysis CKD outpatients from the Osaka Vitamin D Study in Patients with CKD (***OVIDS-CKD***), a prospective observational cohort study in Osaka, Japan [27]. All patients gave written informed consent to participate in the study. The Ethics Committee at Osaka University Hospital approved this study (approval number 07142). Subjects were enrolled from May 2005 to July 2007.

Baseline investigation including laboratory measurements

Data on baseline characteristics, including DM, prior CVD history, and medication were collected through patient records. Prior CVD was defined as a history of stroke, CAD, HF, aortic disease, valvular disease, or PAD. Patients had their BP measured seated in an office. Blood and urine samples were obtained at the enrollment. After a 30-min incubation, blood samples were centrifuged for serum separation and the sera were stored frozen at −80 °C until analysis. Blood chemistry parameters (creatinine, Alb, Ca, and inorganic phosphate) were measured by standard automated techniques. We calculated eGFR according to a Japanese standard formula based on inulin clearance ($194 \times \text{Creatinine}^{-1.094} \times \text{Age}^{-0.287}$ (if female $\times 0.739$)) [28]. Full-length 1–84 PTH was measured by a third-generation assay (Whole PTH, Scantibodies, Santee, CA, USA). The biologically active form of FGF23 (intact FGF23) was measured by a sandwich enzyme-linked immunosorbent assay system (Kainos Laboratories, Inc., Tokyo, Japan). We used this assay because C-terminal FGF23 fragments were reported to be present as circulating FGF23 in serum from patients with end stage renal disease [29]. Levels of serum 1,25D and 25D were measured using a TFB 1,25-dihydroxyvitamin D RIA kit (Immunodiagnostic Systems Ltd., Boldon, UK) and a ^{125}I RIA kit (DiaSorin Inc., Stillwater, MN, USA), respectively. Serum BSAP concentrations were measured by EIA (Osteolinks BAP; Quidel Corp., San Diego, CA, USA). Serum Ca levels were corrected for Alb using the following formula (corrected Ca = total Ca + (4.0 − Alb) × 0.8, if Alb <4.0 g/dL). Urinary protein was measured semiquantitatively with a dipstick test.

Study endpoints

Patients were seen regularly, with the frequency dependent on their conditions and renal function, usually every 1–3 months, and followed prospectively until the study end date (July 1, 2010), death, or loss to follow-up. For the first analysis, we defined the primary endpoint as any fatal/non-fatal cardiovascular event requiring hospitalization before the initiation of dialysis. Patients were censored when starting dialysis therapy. For the second analysis, we did not censor the patients at the start of dialysis but continued follow-up even after the initiation of dialysis. The cardiovascular events included stroke, CAD, HF, aortic disease, and PAD. We also examined deaths as a secondary endpoint. To avoid loss to follow-up, we contacted the patients' physicians by telephone or letter if the patients had changed their hospital.

Statistical analyses

Data are presented as the mean ± SD or the median and IQR, as appropriate. FGF23 and PTH were log-transformed prior to analyses. Differences in variables between the patients with and without cardiovascular events were evaluated by χ^2 tests for categorical variables, and *t*-tests or Wilcoxon rank sum tests for continuous variables. We used binary variables for administration of ACE-I/ ARB, calcium carbonate, and active vitamin D (alfacalcidol and calcitriol). Neither ergocalciferol nor cholecalciferol was available in Japan. In survival analyses, we calculated cumulative cardiovascular event rate curves using Kaplan–Meier analysis followed by log rank test, according to quartiles of serum 25D, PTH, FGF23, and BSAP levels. Since the number of patients without any missing values was 433, we imputed missing data using MI on the basis of the variables of age, sex, DM, prior CVD, eGFR, urinary protein, and the administration of ACE-I/ARB, calcium carbonate, and active vitamin D. We created five MI datasets using the multivariate normal model. We did not impute missing values of urinary protein (15.9%), because of missing not at random (missing in patients with minimal or much urinary protein). In the MI analyses, the analysis of each dataset was carried out separately, and then five sets of estimates (hazard ratios) are combined to generate a single set of estimates [30]. Multiple dichotomous variables were created with urinary protein (−) as a reference. We employed Cox proportional hazards models to estimate the risk of each variable. In multivariable analyses, we adjusted for important clinical parameters reported to be associated with cardiovascular events or mineral metabolism. We tested for interactions between FGF23 and two major CVD risk factors, DM or prior CVD, adding a two-way cross-product term in the multivariable model and using likelihood ratio test. The proportional hazards assumption was tested by creating time-by-covariate interactions for each variable. We also conducted a complete case analysis. Then, we categorized patients into four groups by the median of FGF23 and DM or prior CVD. Graphical analyses were also performed with Kaplan–Meier curves stratified by prior CVD or DM to evaluate qualitative interaction. To assess the incremental contribution of FGF23 to the base model, we computed the reclassification of the predicted 4-year risk for a CVD outcome using the NRI [31,32] and used cross-tabulation to compare the models in 443 patients without missing values who were not censored within 4 years [33]. The base model included the significant risk factors of age, sex, DM, prior CVD, PP, and eGFR. We grouped the predicted probabilities into risk categories of low (<5%), intermediate (5–15%), and high risk (>15%). The statistical test was two-tailed, and $P<0.05$ was considered statistically significant.