Atherosclerosis 236 (2014) 360–365

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Association of vascular endothelial factors with cardiovascular outcome and mortality in chronic kidney disease patients: A 4-year cohort study

Mehdi Rambod [a, 1], Gunnar H. Heine [b, **, 1], Sarah Seiler [b], Elizabeth A. Dominic [d], Kyrill S. Rogacev [b], Rama Dwivedi [c, e], Ali Ramezani [c], Maria R. Wing [c], Richard L. Amdur [f], Danilo Fliser [b], Dominic S. Raj [c, *]

[a] Department of Internal Medicine, Texas Tech University Health Sciences Center, Paul L. Foster School of Medicine, El Paso, TX, USA
[b] Saarland University Medical Center and Saarland University Faculty of Medicine, Internal Medicine IV – Nephrology and Hypertension, Homburg, Saar, Germany
[c] Division of Renal Diseases and Hypertension, The George Washington University School of Medicine, Washington, DC, USA
[d] The George Washington University, Washington, DC, USA
[e] Division of Cardiovascular and Renal Products, Office of New Drugs, US Food and Drug Administration, Silver Spring, MD, USA
[f] Biostatistics Core, The George Washington University, USA

ARTICLE INFO

Article history:
Received 11 April 2014
Received in revised form
23 July 2014
Accepted 25 July 2014
Available online 12 August 2014

Keywords:
sFlt
Placental growth factor
Vascular endothelial growth factors
Survival

ABSTRACT

Background: Angiogenic cytokines fms-like tyrosine kinase-1(sFlt-1) and placental growth factor (PlGF) are associated with increased risk for cardiovascular disease (CVD) in the general population. In this study we examine the association between these vascular endothelial factors and atherosclerosis, cardiovascular outcome, and mortality in chronic kidney disease (CKD) patients.
Methods: Serum level of PlGF and sFlt-1 were measured in 301 patients with CKD, who were followed for up to 4 years. Primary outcomes were CV events and all-cause mortality. Carotid-intima media thickness (CIMT) was used as marker of atherosclerosis. Kaplan–Meier survival curves and the Cox proportional hazard model were used to assess the association of biomarkers and clinical outcomes.
Results: Mean (SD) PlGF and sFlt-1 were 5.45 ng/ml (3.76) and 68.6 (28.0) pg/ml, respectively. During the follow up time, 60 patients (19.9%) experienced CV events and 22 patients (7.3%) died. Compared with low PlGF, patients with PlGF above median level had higher CV events (12.7% vs. 27.2%, $p = 0.002$) and mortality (2.0% vs. 12.6%, $p < 0.001$). The associations of PlGF and sFlt-1 with CV events were not statistically significant in the fully adjusted model. Higher PlGF was associated with greater death risk (HR = 5.22, 95% CI: 1.49–18.33, $p = 0.01$), which was robust to adjustment for sFlt-1 and other risk factors. Elevated sFlt-1 level was also an independent predictor of mortality (HR 3.41, 95% CI: 1.49–9.51, $p = 0.019$).
Conclusion: In CKD patients not yet on dialysis, higher serum level of PlGF and sFlt-1 are associated with increased mortality, but not CV events.

1. Introduction

Patients with chronic kidney disease (CKD) are at increased risk of cardiovascular disease (CVD) and mortality compared to the general population [1]. Traditional CV risk factors such as advanced age, diabetes, smoking, and hyperlipidemia are more prevalent in CKD population. However, compelling evidence shows that non-traditional risk factors such as uremic toxins, endotoxemia, malnutrition, and inflammation play pivotal role in CVD, and poor outcome in CKD patients, at least in part by amplifying the risk of atherosclerosis in CKD patients [2–4].

Vascular endothelial growth factors (VEGF) are a family of endothelial-specific mitogens that have angiogenic properties [5,6]. Placental growth factor (PlGF) is a member of the VEGF family that binds to the VEGF receptor-1 and mediates angiogenesis and

* Corresponding author. Chief Division of Nephrology and Hypertension, MFA-The George Washington University School of Medicine, 2150 Pennsylvania Ave NW, Washington, DC 20037, USA. Tel.: +1 202 741 2283.
** Corresponding author.
E-mail address: draj@mfa.gwu.edu (D.S. Raj).
[1] Contributed equally to the manuscript.

http://dx.doi.org/10.1016/j.atherosclerosis.2014.07.026
0021-9150/

endothelial dysfunction [7]. It was first discovered in the placenta; however, it was later localized to other tissues such as heart and lung. Soluble VEGF receptor 1, also known as soluble fms-like tyrosine kinase-1(sFlt-1), is a splice variant of the VEGF receptor without the transmembrane and intracellular tyrosine kinase domain. It is a potent endogenous antagonist of VEGF and PlGF [8,9]. PlGF-expression within human atherosclerotic lesions is associated with vascular inflammation, thrombus formation, and plaque destabilization [10]. PlGF and sFlt-1 levels are elevated in patients with coronary artery disease and are predictors of adverse outcome [11].

There is emerging interest in angiogenic cytokines in the pathogenesis of CVD in patients with CKD [8]. In the present study, we investigated the association of circulating levels of PlGF and sFlt-1 with markers of atherosclerosis, cardiovascular outcome and all-cause mortality in a prospective cohort of CKD patients with longitudinal follow-up up to 4 years.

2. Methods

CARE FOR HOMe study is an ongoing study of CKD patients from outpatient nephrology clinic in Saarland University Hospital. The study was approved by the local Ethics Committee, and all patients signed written informed consent. Patients were included in this study if they had CKD stage 2, 3, or 4, defined as an estimated glomerular filtration rate (eGFR) between 15 and 90 ml/min/1.73 m^2 according to MDRD equation. Patients younger than 18 years of age, pregnant women (based on self-report), allograft recipients, patient receiving systemic immunosuppressive medication and those with concomitant human immunodeficiency virus infection, clinically apparent infections (defined as C-Reactive Protein (CRP) levels above 50 mg/l, and/or requiring systemic antibiotic therapy), active cancer disease, malignant hematological disorders, and/or acute renal failure (defined as an increase of plasma creatinine $\geq$50% within four weeks) were excluded from study participation.

A standardized questionnaire was used to record a history of smoking, diabetes mellitus status, current medication intake, and cardiovascular co-morbidities. Furthermore, chart review was done to complete and ascertain co-morbidities. Prevalent CVD was defined as a history of myocardial infarction, coronary artery angioplasty and/or stenting and/or coronary bypass surgery, major stroke, carotid endarterectomy and/or stenting, non-traumatic above the knee amputation, or lower limb artery bypass surgery and/or angioplasty and/or stenting.

Patients were categorized as active smokers if they were current smokers or had stopped smoking <1 month prior to participation. Patients with self-reported or physician-reported diabetes mellitus, with a fasting blood glucose level of $\geq$126 mg/dl or with current use of hypoglycemic medication, were categorized as diabetic. Body mass index (BMI) was calculated as weight (kg)/(height (m^2)).

All patients were invited annually for follow-up examinations. The combined end points were as described earlier, which is first occurrence of an atherosclerotic event, the time to decompensated heart failure or death from any cause [12]. All patients were followed up until 31 December 2012.

2.1. Laboratory measurements

Blood samples were obtained under standardized conditions after an overnight fast. Within 15 min, the samples were centrifuged at 4000 r.p.m. for 5 min at room temperature. Supernatants were immediately stored in aliquots at −80 °C until further use. Serum level of intact PTH was measured by second-generation ECLIA (Hoffmann-La Roche, Bale, Switzerland; range of normal values: 15–65 pg/ml), and serum levels of calcium, and phosphate were measured by standard laboratory methods. In all 301 patients, sFlt-1 levels were determined by Quantikine kit (Human sVEGF R1/Flt-1, Cat #: DVR100B, Sensitivity: 1.5–13.3 pg/ml with mean minimum detectable dose of 3.5 pg/ml. CV(%): 2.6 intra-assay precision and 5.5 inter-assay precision). PLGF was measured by sandwich enzyme immunoassay using commercial ELISA kit (R&D Minneapolis, USA). All measurements were done in duplicates. Intra and inter-assay variability were less than 12%.

2.2. Assessment of atherosclerosis

As a marker of systemic vascular atherosclerosis, the intima media thickness of the common carotid arteries was measured (CIMT). With the subject in the supine position and the head slightly extended and turned to the opposite direction, the distal common carotid artery and the carotid bulb were identified with longitudinal scanning. IMT was defined as the distance between the leading edges of the lumen interface and the media-adventitia interface of the far wall. Three representative CIMT measurements were performed on both sides in the far wall of the common carotid arteries at 1.0, 2.0, and 3.0 cm proximal to the bifurcation, and these six CIMT readings were averaged to give the mean common carotid CIMT.

2.3. Statistical analysis

Data management and statistical analysis were performed with SPSS version 17. p values <0.05 was considered statistically significant. Categorical variables are presented as percentage of patients and were compared using the Chi-2 test. Continuous data are expressed as mean ± standard deviation, or median (quartile 25–75) for variables with skewed distribution. A T-test or Mann–Whitney U test were used to compare mean or median of variables in the two groups of patients with PlGF above vs. below median level. The correlation between continuous variables was assessed by Pearson correlation testing or Spearman's rho, whichever was appropriate. The most appropriate PlGF transformation for fitting the PlGF vs. eGFR association was investigated based on minimizing the Akaike Information Criterion (AIC) [13]. Multivariate linear regression model, with stepwise approach, was used to examine the predictors of PlGF. Variables that were used in the stepwise model included age, sex, eGFR, creatinine, Cystatin C, albuminuria, LDL-C, HDL-C, Triglyceride, WBC, PTH. Cox proportional hazard model, Kaplan–Meier survival curves, and log-rank test were used to assess the association of PlGF and clinical outcomes. First, we used Cox proportional hazard model without adjusting for confounders to examine the hazard ratio (HR) of cardiovascular outcome or mortality in CKD patients with PlGF above median level versus those below the median level as reference group. We used the same approach for association of sFlt-1 and outcome. In model 1, we included both PlGF and sFlt-1 to examine the proportional association of each variable with outcomes. Model 2 was adjusted for age and sex. Model 3 was adjusted for BMI, diabetes, smoking, SBP, and LDL-C as well as age and sex. In model 4, albuminuria and eGFR were also added to the previous adjustors.

3. Results

Mean patient age was 65.7 ± 11.8 years. Sixty one percent of participant were male ($n = 184$), 39% ($n = 118$) had diabetes, 32.7% ($n = 98$) had known cardiovascular disease, and 11.3% ($n = 34$) were smokers. CKD stages 2, 3, and 4 encompassed 16.6% ($n = 50$), 60.8% ($n = 183$), and 22.6% ($n = 68$) of the total cohort, respectively. Mean