Prognostic Analysis of Uremic Factors of Cardiovascular Risk, Including FGF-23, Klotho and Sclerostin, in Assessing the Progression of Vascular Calcification in CKD Patients: A Prospective Observation

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Abstract

Background: Cardiovascular Calcification (CVC) is a major contributor to the high incidence of Cardiovascular Events (CVE) in Chronic Kidney Disease (CKD). Early CVC markers are actively studied now in CKD for cardio-renalprotective strategy optimization. We have conducted a prospective comparative analysis testing the following factors: FGF-23, Klotho, sclerostin, phosphate, parathyroid hormone serum levels, estimated Glomerular Filtration Rate (eGFR), central systolic Blood Pressure (BP) levels, as independent determinants of CVC.

Materials and methods: A total of 131 CKD stage 2-5D patients were included. Serum FGF-23, Klotho, and sclerostin levels were measured by ELISA. Augmentation indices, central (aortic) BP (by <SphygmoCor>), valvular calcification score (by Echocardiography), and aortic calcinosis score (by abdominal aorta radiography), were performed. The observation period was 2 years.

Results: According to Spearman’s correlation analysis, the percentage of calcification increase and the change in serum Klotho level were most related. According to ROC analysis, a decrease in Klotho serum level by 50 units or more is a significant predictor of an increase in aortic calcification by 50% or more with a sensitivity of 84% and a specificity of 75%. Using logistic regression analysis, it was found that Klotho serum level <632 pg/L will predict eGFR below the median value of 48 ml/min/1.73 m² with a sensitivity of 84.5% and a specificity of 76.5%. Wherein, OR=17.477 (CI 95% 8.046-37.962, p<0.001).

Conclusion: The factor most associated with CVC is Klotho. A decrease in Klotho serum level is a significant predictor of increase in aortic calcification. In addition, initial Klotho serum level is a predictor of eGFR level over 2 years period.

Keywords: Chronic kidney disease; Fibroblast growth factor-23 (FGF-23); Serum Klotho (sKlotho); Sclerostin; Cardiovascular calcification; Cardiac remodeling

Introduction

It is known that patients with Chronic Kidney Disease (CKD) have a high burden of cardiovascular morbidity and mortality and are more likely to die from Cardiovascular Events (CVE) than to develop end-stage renal failure [1-3]. The traditional risk factors for cardiovascular disease, that is, arterial hypertension, dyslipidemia and diabetes mellitus, are very common in the population of patients with CKD. However, in recent years it has been proven other cardiovascular risk factors that are ‘uremia specific’ can contribute to the increased incidence of CVE in CKD [3-5]. These factors include, among others, changes in the levels of morphogenetic proteins (fibroblast growth factor-23, Klotho) and glycoprotein sclerostin that participate in the maintenance of bone and mineral homeostasis [6,7]. Fibroblast Growth Factor (FGF)-23 is a well known bone-derived hormone that regulates phosphate homeostasis and vitamin D metabolism. Early rise in plasma FGF-23 concentration in the course of CKD compensates the inability of the kidneys to excrete an adequate amount of phosphate and so it is an adaptive early CKD mechanism [6-9], however, at the later stages of CKD, a significantly increased FGF-23 level is becoming independently correlated with Left Ventricular Hypertrophy (LVH), endothelial dysfunction, and increased cardiovascular mortality [10,11], and can be considered as a novel early risk factor for CVE progression. Increased FGF-23 production in CKD is closely associated with Klotho depletion [6-13]. Interesting, Klotho is considered as a protein of youth and life [14] Membrane-bound Klotho is expressed in the renal tubules and functions as an obligate co-receptor for FGF-23 so it is involved in FGF-23 mediated phosphorytia [15]. While soluble Klotho (Klotho) is present in blood, urea and cerebrovascular fluid and has multiple systemic biological functions, including regulation of nitric oxide production by endothelium [6,16], maintenance of endothelial integrity and permeability [17], and repression of intracellular signals of insulin and insulin-like growth factor 1, which is responsible for the life expectancy in mammals [14]. Klotho has been demonstrated ameliorated vascular endothelial dysfunction and delayed vascular calcification [16]. Opposite, low serum Klotho levels have been reported to be associated with an increased incidence of CVE [6,12] and all-cause mortality [6,18].

Sclerostin is another emerging factor, secreted primarily by osteocytes, that can contribute to CVC in patients with CKD.
reports suggest that high serum sclerostin levels may reflect a reduced bone metabolism and may be useful as a marker for low-turnover bone disease in advanced CKD [19]. As an antagonist of Wnt/β-catenin signaling pathway, which is involved in vascular biology, sclerostin may be involved in vascular calcification [20]. It has been reported that sclerostin is also associated with clinical outcomes, but results are conflicting [21]. Importantly, since Klotho, FGF23 and sclerostin are closely biologically interrelated, it is difficult to ascribe dismal clinical outcome to one of these factors only, since studies assessing all these factors together, in parallel are scarce. We have conducted a comparative prospective multifactor and ROC analysis to assess the prognostic role of the system of novel biomarkers such as soluble Klotho, FGF-23, and sclerostin levels, and several traditional CKD risk factors such as estimated Glomerular Filtration Rate (eGFR), phosphate and Parathyroid Hormone levels (PTH), as well as some general factors-central Blood Pressure Level (CVP), as independent determinants of CVC progression in a Russian cohort of CKD patients.

Materials and Methods

Study design

A single-center, prospective, ROC and multivariate analysis were conducted. Eligible subjects were recruited from the outpatient department of nephrology clinic at the Sechenov University (during February to August 2016). The protocol of the study was approved by the Ethics committee of the Sechenov First Moscow State Medical University (protocol № 09-16, 15.09.2016). All patients provided written informed consent for the participation in the study that was conducted in accordance with the Declaration of Helsinki. The observation period was 2 years.

Study population

Adult patients were eligible to participate in the study if they had CKD 2-5D, according to KDIGO 2012 guidelines [22]. The exclusion criteria were as follows: diabetes mellitus, systemic autoimmune diseases, age less than 18 or more than 65 years, severe chronic heart failure (III-IV class NYHA), severe refractory stable angina (III-IV class) or acute coronary syndrome, infections, pregnancy, malignancy, arterial hypertension >180/100 mm Hg, Body Mass Index (BMI) less than 19 or more than 30 kg/m², proteinuria >1 g daily, history of kidney transplantation, immunosuppressive therapy. A healthy control population with normal kidney function and no albuminuria or known cardiovascular disease was also recruited. Data on demographic characteristics, past medical history, current medications and blood samples were collected for all subjects at the time of enrollment and end of study.

Biochemical assessments

Blood samples were collected in the morning after at least 8-hour fasting and centrifuged for 15 minutes at 3,000 rpm. The obtained serum was stored at -80°C. Serum Klotho (IBL-Takara 27998-96Well), FGF-23 (Merck Millipore MILLENZFGF-23-32K), and sclerostin (Biomedica, Vienna, BI-20492) levels were measured at the moments of screening and end of the study using ELISA kits in the certified laboratory at the University, according to the manufacturers’ protocols. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [23-26].

Cardiovascular imaging

The Stiffness (Augmentation) Indices (AI), Pulse Wave Velocity (PWV), and central (aortal) Blood Pressure (BP) were measured using a non-invasive device (SphygmoCor 2000; AtCor Medical, Australia). Arterial hypertension was diagnosed if the central systolic blood pressure was more than 130 mmHg and the central diastolic blood pressure was more than 90 mmHg [23]. Cardiac (valvular) calcification score (CVCVS) and left ventricular myocardium mass index (LVMMI) were measured using bi-dimensional echocardiography. CVCVS was evaluated by a semiquantitative scale [22,24]. Left ventricular hypertrophy (LVH) was established if LVMMI was more than 95 g/m² in females and more than 115 g/m² in males [25]. All the patients also underwent plain radiography of the abdominal aorta to assess aortic calcification score (scale from 1 to 24 points, by Kaupplia method) [22,26].

Statistical analysis

Standard descriptive statistics with calculated median (interquartile range), mean ± standard deviation, or frequencies n (%) were used to assess the baseline characteristics of the study population. Logistic regression was used for multivariate analysis. The multivariate model was fitted with percent of aortic calcification as the outcome variables, and mean delta FGF-23, delta Klotho, delta sclerostin, delta eGFR, delta phosphorus, and delta PTH in serum, and delta Central Systolic BP (CSBP) levels, as the exposure variables. Spearman’s correlation coefficient was used to evaluate the relationships between the variables. ROC analysis was used to evaluate the prognostic characteristics of the regression models. In univariate and multivariate analysis of associations, the odds ratios (OR) and 95% Confidence Intervals (CI) were calculated. Two-tailed P-values were considered statistically significant at <0.05 level. All statistical analyses were performed using SPSS software version 21.0 (Chicago, Illinois, USA).

Results

Patients

We screened 540 patients with non-diabetic, non-autoimmune CKD stage 2-5D. From them, 131 patients (65 males and 66 females) with the mean age of 41.1 (20.3-65.2) years were enrolled in the study. The main causes of CKD were chronic glomerulonephritis, tubulointerstitial nephritis, and polycystic kidney disease. Baseline demographic and clinical characteristics of patients, according to stage of CKD, are presented in Table 1. No significant differences were observed between the groups (CKD stages) for age, gender, BMI, diastolic BP, proteinuria levels, proportions of etiologic causes of CKD. At the same time, we can see a statistically significant increase in the CSBF level, cardiovascular co morbidities frequencies, as well as increase of serum levels of phosphate, PTH, erythrocyte sedimentation rate, alkaline phosphatase along with advances of CKD stages (Table 1). In addition it was observed increased cardiovascular calcification degree ranging from an augmentation (stiffness) index of blood vessels to a calcification of heart valves and abdominal aorta as well as an increase in myocardial remodeling (LVMMI) as the CKD stage increases (Table 1 supplementary data).

Control group

A total of 30 subjects without CKD were included in the study in order to examine the relationship between Klotho, FGF-23, sclerostin serum levels in non-CKD patients. These subjects were matched with the CKD patients’ group by age and sex, and were scheduled for echocardiography and sphygmography. Main demographic and clinical characteristics of control subjects are presented in Table 2S (supplementary data). We also analyzed changes in key determinants over a 2-year observation period (Table 2). Next, we calculated the
percentage from the initial of dependent variables (calcification gain) for each calcification model (aortic calcification, cardiac (valvular) calcification, aortic calcification). Two models of calcification were studied factors and the increase in calcification (the percentage of aortic calcification change and delta) (Table 3). According to Spearman`s correlation analysis, the increase in calcification (augmentation index -6, 25(0.0; 13,22)%. Thus, a significant calcification gain over the 2-year observation period was considered to be 50%.

Next, we investigated the relationship between the change in studied factors and the increase in calcification (the percentage of calcification change and delta) (Table 3). According to Spearman`s correlation analysis, the increase in calcification (augmentation index delta, percentage of augmentation index change and aortic calcification delta, percentage of aortic calcification change) and serum Klotho delta were most associated Sclerostin and FGF-23 were associated with calcification to a some lesser extent (Table 3). According to ROC analysis, a decrease in Klotho serum level by 50 units or more was a significant predictor of an increase in aortic calcification by 50% with a sensitivity of 84% and a specificity of 76% (Figure 1). ROC analysis of the predictive value of Klotho level in relation to aortic calcification. These data are confirmed by the results of multivariate analysis (logistic regression). The factors most associated with aortic calcification, according to multivariate analysis, were Klotho and eGFR (Table 4).

However, in univariate analysis of this pair of predictors, only Klotho was statistically significant-OR=0.986 (CI 95% 0.977-0.995, p=0.026), while for eGFR-OR=1.020 (CI 95% 0.973-1.068, p=0.418). Considering the results of multivariate and univariate analysis, we evaluated how eGFR changes depending on Klotho initial level. For this, we previously identified the median of eGFR for dividing the general group of patients into subgroups above and below the median as a measure of the central tendency of the presence of a normal distribution The median of eGFR was 48.0 (21.0-88.0) ml/min/1.73 m². According to logistic regression analysis, the initial Klotho level was a predictor of eGFR level in dynamics with a sensitivity of 79.8 and a specificity of 84%.
Correlation analysis | Klotho delta | phosphorus delta | PTH delta | eGFR delta | sclerostin delta | LnFGF-23 delta | sBP delta
--- | --- | --- | --- | --- | --- | --- | ---
The percentage of augmentation index change | r | -0.830** | 0.227* | 0.360** | -0.412** | 0.473** | 0.448** | 0.369**
p | <0.001 | 0.026 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001
Augmentation index delta | r | -0.801** | 0.189 | 0.355** | -0.405** | 0.485** | 0.423** | 0.325**
p | <0.001 | 0.066 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001
The percentage of cardiac (valvular) calcification change | r | -0.844 | -0.113 | 0.150 | -0.149 | 0.152 | 0.028 | 0.052
| p | 0.068 | 0.404 | 0.264 | 0.268 | 0.258 | 0.842 | 0.702
Cardiac valvular calcification delta | r | -0.152 | -0.035 | 0.098 | -0.056 | 0.097 | -0.036 | 0.075
| p | 0.140 | 0.733 | 0.342 | 0.586 | 0.354 | 0.738 | 0.469
The percentage of aortic calcification change | r | -0.601** | 0.046 | 0.203 | -0.258* | 0.469** | 0.506** | 0.317*
| p | <0.001 | 0.725 | 0.113 | 0.043 | <0.001 | <0.001 | 0.012
Aortic calcification delta | r | -0.547** | -0.014 | 0.298** | -0.238* | 0.371** | 0.257* | 0.271**
| p | <0.001 | 0.894 | 0.003 | 0.019 | <0.001 | 0.014 | 0.008

Table 3: Spearman’s correlation analysis. the increase in calcification (augmentation index delta, percentage of augmentation index change and aortic calcification delta, percentage of aortic calcification change) and serum Klotho delta were most associated. Sclerostin and FGF-23 were associated with calcification to some lesser extent. pg/L. Using logistic regression analysis, it was found that a Klotho level <632 pg/L is predict eGFR below the median value of 48 ml/min/1.73 m² with a specificity of 84.5% and a specificity of 76.5%. Whereas, OR=17.477 (CI 95% 8.046-37.962, p<0.001) AUC fits to 0.850 ± 0.031 (CI 95% 0.790-0.911, p<0.001), which reflect to enough high quality predictive model.

Discussion
Progressive deterioration of kidney function in CKD is associated with different disorders that are similar to those in general aging [2,4]. However, in CKD they develop significantly more rapidly, particularly in cardiovascular system [2-14]. Previous experimental and epidemiologic studies suggested hyperphosphatemia, hyperparathyroidism, and vitamin D deficiency as emerging cardiovascular risk factors in CKD patients [26,27]. Surprisingly, in interventional trials, intake of phosphate binders, cinacalcet, or active vitamin D did not exert a consistently beneficial effect to reduce cardiovascular event rates [28-30]. The accumulating evidence of recent years suggests FGF-23, Klotho and sclerostin (key regulators of mineral metabolism) can determine cardiovascular and kidney dysfunction in patients with CKD [7-21]. In our work we have attempted to study the combined and comparison effects of these novel factors as well as of traditional markers, on the parameters of stiffness/calcification of heart and blood vessels in CKD
induces chronic inflammation and oxidative stress in injured vessels 
uptake by VSMC [31], indirectly influences the vascular system and 
evidence that increased FGF-23 in CKD induces active phosphate 
and CV outcomes in CKD patients is now actively studied. There is 
Similar to Klotho, the relationships between the levels of FGF-23 
clinical data [6-14]. At least five possible mechanisms of Klotho`s anti-
calcification can be understood in light of recent experimental and 
Thus, Klotho's serum level was stronger than the other studied factors, 
on decreased eGFR level (OR=0.992 (CI 95% 0.990-0.995, p<0.001). Therefore, 
level by 50 units or more is a significant predictor of an increase in 
association of FGF-23 with calcification of the heart and blood vessels 
was noted to a lesser extent than with Klotho. At the same time, there is 
reason to believe that FGF-23 predominantly induces the development of 
cardiomyocyte hypertrophy and heart failure and to a lesser extent 
affects the blood vessels [10,36].

Less understood yet is the role of sclerostin in processes of 
calcification in CKD. The results of correlative analysis obtained in 
this work are in agreement with observations of other authors who demonstrated protective effect of sclerostin on calcification in 
CKD [21-37]. The accumulating data of recent years more lean that 
overexpression of Wnt signaling pathway inhibitors in calcifying 
vasculature (advanced carotid plaques and calcified aortas) might be 
vasculoprotective and anti-calcific [21]. At the same time the recent 
experimental rat model also showed the overexpression of secreted 
Frizzled-related proteins (another group of Wnt pathway inhibitors) 
in the late but not the early stages of vascular calcification. Our analysis 
demonstrated that the higher circulating sclerostin levels in patients 
with CKD were independently associated with a lower risk for vascular 
calcification, but its anti-calcification effect was significantly less 
expressed than Klotho’s effect and it may manifested in more advanced 
CKD stages. Thus, our data permit to suggest that sclerostin plays a role 
of a protective factor, which addressed to prevent pathogenic effects 
of decreased Klotho and increased FGF23 levels, and allows for some 
time to maintain a compensatory balance in FGF-23/Klotho/sclerostin 
system in CKD progresses.

Conclusion

According to our data, despite the fact that all three studied factors 
begin to change from the early CKD stages, the most pronounced effects 
associated with CVC were found for Klotho. The results of our work 
demonstrate that the studied regulatory proteins (FGF-23, Klotho, 
sclerostin) can be considered as a discrete system of factors influencing 
CVC risk, with Klotho playing more early and important role in CVC. 
The high risk of CVC in patients with CKD is determined by a joint 
effect of all these novel factors, which are connected consequentially 
as CKD advances and combined with traditional CKD risk factors. It 
is noteworthy that these factors, which are initially caused by CKD, 
rapidly become independent from it and potentiate one another led 
to substantial activation of CVE and uraemia progression in CKD 
patients.

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Compliance with Ethical Standards

The study was approved by the Institutional Review Board of the Sechenov 
First Moscow State Medical University (Protocol No 09-16, 15.09.2016). All the 
patients provided written informed consent. All the described procedures were 
demonstrated in accordance with the Helsinki Declaration.

References


Figure 3: Using logistic regression analysis, it was found that a Klotho level <32 pg/L predict eGFR below the median value of 48 ml/min/1.73 m² with a 
sensitivity of 84.5% and a specificity of 78.5%. Wherein, OR=17.477 (CI 95% 
0.804-37.962, p<0.001) wherein, according to ROC analysis, the AUC fit to 
8.046-37.962, p<0.001), which indicates enough high 
level of prediction of the prognostic model.

The central role of Klotho in the underlying mechanisms of 
calcification can be understood in light of recent experimental and 
clinical data [6-14]. At least five possible mechanisms of Klotho`s anti-
calcification effect were established:

- Action as a phosphaturic hormone (as a co-receptor for FGF- 
23) [15];
- The preservation of GFR [6,13];
- Effect on vascular smooth muscle cells (VSMC) by lowering 
their active phosphate uptake (that, is believed, to be induced 
by excess of FGF-23) [31];
- The ability to inhibit of Wnt signaling pathway [32];
- Suppression of TNF-β-induced expression of intracellular 
adhesion molecule-1 and vascular cell adhesion molecule-1, 
attenuates NF-kappaB activation, and reverses the inhibition of 
eNOS phosphorylation by TNF-alpha [33], that suggest that Klotho 
protein is a strong protector of vascular endothelium acting via 
inhibition of endothelial inflammation [33,34].

Reduction of serum Klotho levels impairs these protective effects. 
Similar to Klotho, the relationships between the levels of FGF-23 
and CV outcomes in CKD patients is now actively studied. There is 
evidence that increased FGF-23 in CKD induces active phosphate 
uptake by VSMC [31], indirectly influences the vascular system and 
induces chronic inflammation and oxidative stress in injured vessels

[34,35]. In 2012, Munoz Mendoza et al. [34] discovered that higher 
FGF-23 levels are associated with IL6, CRP, and TNFα in predialysis 
CKD patients. One year later FGF-23 was found to strongly correlate 
to hsCRP in hemodyalisis patients [35]. On the other hand increased 
production of FGF-23 is induced by phosphorus and decreased Klotho 
levels in CKD [6]. Thus, it may be hypothesized that pathways involving 
FGF-23, Klotho, phosphorus can determine chronic inflammation, 
obstructive stress, endothelial dysfunction and vascular calcification 
[6-35]. However, it should be clarified that according to our data, the 
association of FGF-23 with calcification of the heart and blood vessels 
was noted to a lesser extent than with Klotho. At the same time, there is 
reason to believe that FGF-23 predominantly induces the development of 
cardiomyocyte hypertrophy and heart failure and to a lesser extent 
affects the blood vessels [10,36].


