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Original Article

Assessment of the relationship between serum soluble Klotho and carotid intima–media thickness and left ventricular dysfunction in hemodialysis patients

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Abstract

Background: The aim of our study was to assess the relationship between soluble Klotho (s-Klotho) and carotid intima–media thickness (CIMT) and left ventricular (LV) dysfunction in hemodialysis (HD) patients.

Methods: This is a cross-sectional study conducted on 88 patients with end-stage renal disease on regular HD. Serum levels of calcium, phosphorus, parathyroid hormone, and C-reactive protein were measured. The serum levels of s-Klotho and fibroblast growth factor-23 (FGF-23) were measured using an Enzyme linked immunosorbent assay (ELISA) kit. Echocardiography and measurement of CIMT were also conducted. The studied patients were divided according to the median s-Klotho level into 2 groups: patients with low s-Klotho (Group I) and patients with high s-Klotho (Group II).

Results: Mean value of s-Klotho was significantly low in HD patients compared to controls ($P = 0.001$), and mean value of FGF-23 was significantly high in HD patients compared to controls ($P = 0.001$). The mean values of parathyroid hormone, FGF-23, and phosphorus were significantly high in Group I compared to Group II, whereas the mean value of serum calcium was significantly low in Group I compared to Group II. The mean values of CIMT, LV mass (LVM), LVM index, and LV ejection fraction (LVEF) were high in Group I compared to Group II. Patients with low s-Klotho had significantly more coronary artery disease (CAD). In a regression analysis of s-Klotho with different markers of cardiovascular diseases, s-Klotho showed significant association with CIMT, LVEF, and CAD, but not with LVM and LVM index.

Conclusion: The present study showed that patients with a low s-Klotho were more often associated with increased CIMT, LV dysfunction, and CAD, and it seems that there was independent association between s-Klotho and CIMT, LVEF, and CAD.

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Introduction

Chronic kidney disease (CKD) is associated with increased levels of parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF-23) and hypocalcemia, hyperphosphatemia, bone disease, vascular calcification, and cardiovascular morbidities collectively referred to as CKD-mineral and bone disorder [1–3]. Recent reports suggested that increased levels of FGF-23 and decreased levels of soluble Klotho (s-Klotho) are common manifestations of CKD that develop earlier than increased levels of phosphate (Ps) or PTH [4,5].

A transmembrane (TM) protein known as soluble α-Klotho (s-Klotho) is primarily produced in the kidney distal tubular cells [6]. Soluble α-Klotho acts as a coreceptor for the bone-derived protein FGF-23 [7,8]. Regulation of both renal handling of Ps and renal synthesis of calcitriol needs a cofunction of both FGF-23 and TM-Klotho [9]. Soluble α-Klotho is the circulating protein resulting from the shedding of the extracellular domain of TM-Klotho operated by 2 metalloproteinases of the A disintegrin and metalloproteinase domain–containing protein (ADAM) family: ADAM10 and ADAM17 [10]. In particular, s-Klotho inhibits the sodium-Ps cotransporter NaPi2a expression in the proximal tubules, thus generating a phosphaturic effect additive to and independent of FGF-23 [11–13], and activates the ion channel TRPV5 in the distal tubules, thus increasing tubular reabsorption of calcium (Ca) [14]. Therefore, α-Klotho, with its TM and soluble forms, is deeply involved with the physiological regulation of mineral metabolism [15].

CKD is a common risk factor for cardiovascular diseases (CVD) such as coronary artery disease (CAD), cerebrovascular stroke, peripheral vascular disease, and heart failure [16]. Although part of CVD burden in patients with CKD is related to traditional risk factors, CKD-associated disturbance in Ca–Ps homeostasis plays a crucial role as well [2]. Recent studies showed that FGF-23 and its coreceptor s-Klotho have an important role in Ca–Ps homeostasis, and they could be the missing link in the detrimental relationship between CKD and CVD [17].

Given the strong cardioprotective effects of Klotho demonstrated in preclinical studies, the present study aimed to assess the association between s-Klotho and carotid intima–media thickness (CIMT) and left ventricular (LV) dysfunction in patients with end-stage renal disease (ESRD) on regular hemodialysis (HD). It was hypothesized that low levels of s-Klotho are associated with a larger burden of CVD in these patients.

Methods

Study population

This is a cross-sectional study conducted on 88 patients with ESRD on regular HD of at least 6-month duration. All patients have been attending the nephrology dialysis unit in the Theodor Bilharz Research Institute, Giza, Egypt. All patients received 3 sessions of HD/wk, each of 4-hour duration using a polysulfone dialyzer (Fresenius, St. Wendel, Germany) with surface area of 1.4–1.6. Patients with advanced congestive heart failure [defined clinically by elevated jugular venous pressure, ascites, peripheral edema, and shortness of breath at rest and LV ejection fraction (LVEF) of < 30% in echocardiography requiring hospital-based support, a heart transplant, or palliative care according to guidelines of the American College of Cardiology/American Heart Association] [18], sepsis, or malignancy were excluded from the study. Informed written consent was obtained from all participants. The study protocol was approved by the institute ethics committee, and the study was performed in accordance with the Declaration of Helsinki.

The studied patients were divided according to the median s-Klotho level (cut point, 476 pg/mL) into 2 groups: patients with low s-Klotho < 476 pg/mL (Group I, $n = 44$) and patients with high s-Klotho > 476 pg/mL (Group II, $n = 44$).

A control group consisting of 28 normal individuals was used to give our reference values for s-Klotho, FGF-23, CIMT, and echocardiographic findings. There were 17 men and 11 women with a mean age of 53.3 ± 16.2 years, with normal renal function and no evidence of acute or chronic underlying disease.

Each patient underwent a thorough history and clinical examination. Demographic characteristics and coexisting conditions such as atherosclerotic CAD diagnosed by electrocardiography and coronary angiography within 3 months of enrollment in the study were collected.

Fasting blood samples were collected from the patients before the HD session, immediately centrifuged, aliquoted in vials, and stored at −60°C until the time of analysis. Thawing the test samples was carried out at a low temperature by mixing them completely before measurement. Routine examinations included complete blood analysis, kidney function tests (serum urea, creatinine, sodium and potassium, and uric acid), random blood sugar, serum electrolytes, lipid profile, and serum albumin. Serum levels of Ca, Ps, PTH, and C-reactive protein were measured.

Serum levels of s-Klotho and FGF-23 measurement

The serum levels of s-Klotho were measured using an Enzyme linked immunosorbent assay (ELISA) system (Immuno-Biological Laboratories, Gunma, Japan) [19], and this assay detects circulating s-Klotho using 2 monoclonal antibodies that specifically recognize the extracellular domain of Klotho, with a lower limit of detection of 6.15 pg/mL and the intra-assay and interassay coefficients of variation of < 10%. The serum levels of FGF-23 were measured using a commercial sandwich ELISA kit (Kainos Laboratories, Inc., Tokyo, Japan) [20] that uses a 2-site ELISA for the full-length molecule. Two specific murine monoclonal antibodies recognize the biologically active FGF-23, with a lower limit of detection of 3 pg/mL and interassay and intra-assay coefficients of variation of < 5%.

Residual renal function estimation

Residual renal function (RRF) was estimated by calculating glomerular filtration rate expressed in mL/min/1.73 m^2. Glomerular filtration rate was estimated as the mean of urea and creatinine clearance using 24-hour urine collections and the mean of the post- and pre-HD plasma urea and creatinine. RRF was considered zero in patients with a urinary output < 100 mL/24 h [21]. Kt/V was used to assess dialysis adequacy [22].

Echocardiography

After the HD session, each patient underwent echocardiography to determine LV mass (LVM), LVM index (LVMI), and LVEF.