BIOMARKERS - FGF-23 AND A-KLOTHO IN HEMODIALYSIS PATIENTS WITH SECONDARY HYPERPARATHYROIDISM

Svetla Staykova

Department of Internal Diseases, Faculty of Medicine, Medical University of Varna

ABSTRACT

Chronic kidney disease (CKD) has been shown to impair the metabolism of a number of minerals, resulting in bone damage, vascular wall calcifications, functional disorders, and significant mortality.

Renal osteodystrophy in this disease is characterized by histological bone abnormalities and altered rate of bone transformation (increased in fibrous osteitis or decreased in adynamic bone disease), pathological mineralization (osteomalacia), and bone loss. Secondary hyperparathyroidism (SHPT) is associated with fibrosis osteitis, early build-up of phosphorus (responsible for excess production of fibroblast growth factor-23 (FGF-23) by the bone tissue), decreased production of calcitriol by the kidneys and hypocalcemia. Other bone debilitating factors include acidosis, chronic inflammation, food deficiency, and iatrogenic complications.

It has been proven that with the progression of CKD and the reduction of glomerular filtration, the activity of FGF-23 is increased, which leads to inhibition of alpha-1 hydroxylase and to decreased levels of 1,25 dihydroxyvitamin D.

FGF-23 comes predominantly from the bones, while α-Klotho - from the kidneys, and together they play different important roles to maintain mineral metabolism. CKD is associated with significant increases in FGF-23 concentrations and inhibition of α-Klotho.

Hyperphosphatemia, which correlates with endothelial dysfunction and elevated FGF-23, with an untimely treatment, results in pruritus, bone pain, anemia, cardiovascular complications, disability, and reduced survival rate.

The association between protein-related uremic toxins and FGF-23 is a challenge motivating the study of bone biomarkers. Scr Sci Med. 2018;50(1):36-40

Keywords: *CKD, hypophosphataemia, FGF-23, alpha-Klotho*

Address for correspondence:
Svetla Staykova
Faculty of Medicine
Medical University of Varna
55 Marin Drinov St
9002 Varna
e-mail: svetlastaykova@abv.bg

Received: November 28, 2017
Accepted: March 30, 2018

INTRODUCTION

The aim of our study is to determine the level of FGF-23, α-Klotho in serum in patients on hemodialysis (HD), as well as a correlation analysis showing a positive relationship between serum concentrations of fibroblast growth factor-23 (FGF-23) and changes in the concentrations of calcium and phosphorus, but not 25(OH)D.

Our study proves that the levels of α-Klotho and FGF-23 are decreased in the parathyroid glands of patients with chronic kidney disease (CKD).

Low serum α-Klotho levels are most commonly associated with endothelial dysfunction in patients with CKD (1). Alpha - Klotho urine levels are more sensitive biomarkers than serum α-Klotho levels in patients with CKD.

Small molecule metabolites play an important role in biological systems and are attractive biomarkers for the understanding of CKD (2).

According to D. Yonova and P. Dukova (3), for the monitoring of bone transformation in patients with uremia, specific and sensitive serum biochemical markers are required. The ideal marker should be unique to the bone and reflect the overall skeletal activity. Some of them - osteocalcin, bone-specific alkaline phosphatase, and procolagen-I-propeptide are indicators of osteoblast activation, and others - the dioxinpyridinoline, hydroxyproline and tartrate acid phosphatase - to activate osteoclasts.

The hyperphosphatemia that is demonstrated [it is subclinical to glomerular filtration rate (GFR) <30 ml/min] is a leading cause of secondary hyperparathyroidism. Phosphorus induces parathyroid hormone (PTH) secretion by three mechanisms: direct stimulation of the parathyroid glands; induction of hypocalcemia (precipitation of calcium, such as CaHPO4 and decreased calcium release from bone); and increased activity of FGF-23 (this leads to inhibition of alpha-1 hydroxylase and lowering the levels of 1,25 dihydroxyvitamin D) (4).

There is a gradual increase in bone resorption associated with reduced bone formation -impaired bone mineralization. Bone expression of sclerostin and parathyroid hormone receptor 1 increases in the early stages, and that of FGF-23 and phosphorylated β-catenin in the late stages of CKD (20). Sclerostin and FGF-23 are with different immunohistochemical localization, indicating that they are produced by various osteocytes. There is a positive correlation between serum concentrations and bone expression of FGF-23 (5).

H. Fujii and N. Joki (6) assume that other specific factors play an etiopathogenetic role and highlight FGF-23, which is independently associated with remodeling in the heart. The slow development of vascular calcifications and remodeling in the heart is characteristic of cardiac pathology due to CKD with impaired bone mineral metabolism. There is also the arterial calcification of acute heart disease, most often causing cardiac infarctin, angina pectoris, and peripheral vascular disease. Studies have shown that serum levels of FGF-23 and its α-Klotho co-receptor expression in parathyroid tissue are significantly increased by reducing renal function. The association between protein-related uremic toxins and FGF-23 is a challenge motivating the study of bone biomarkers.

MATERIAL AND METHODS

Two groups of patients were studied: with high iPTH (55 patients - 22 men and 33 women, mean age 58.9), and with a normal value of iPTH (51 patients of which: 19 men and 32 women with long-term hemodialysis treatment 2-7 years, at the same age, having HD three times a week for 4 hours), for both groups the levels of FGF- 23 and α-Klotho in serum are defined.

For the study of α-Klotho levels, the DuoSet ELISA Human Klotho Kit was used, and for the level of FGF-23 - multimatrix ELISA for quantification of human FGF-23 (C-terminal) in human serum by sandwich ELISA. The results were obtained by measuring the optical density of the resulting solutions, based on which mathematical formulas draw standard curves showing the corresponding serum concentrations.

For statistical analysis of the data a specialized package was used - StatSoft Inc., USA, STATISTICA Manual (Data analysis software system), Version 10.0, 2010.

For significance level, p=0.05 was selected. This is the probability of committing a first-order error, namely, to reject the null hypothesis when it is true.

For the purposes of this study, the following statistical methods were applied:

1. Descriptive statistical analysis - the frequency distribution of the examined signs is presented in tabular form, broken down by study groups, averages and standard deviations, 95% confidence intervals of change of the mean values.
2. Student's t-test (t-criterion) for two independent samples - to detect a statistically significant dif-

ference in the mean values of a factor in two patient groups.

3. Student's t-test (t-criterion) for two paired samples - to detect a statistically significant difference in the mean values of a factor before and after treatment.

RESULTS AND DISCUSSION

According to M. Kuro-O and O. W. Moe (7), FGF endocrine factors and Klotho genetic families are complex systems of common origin with the endoskeleton. FGF-23 is predominantly derived from bones, and α-Klotho from the kidneys, and together they play different important roles to maintain mineral metabolism.

One unique feature of FGF-23 is the need for Klotho as an imperative co-receptor. The FGF-23 and Klotho system is an endocrine axis necessary to maintain phosphorus homeostasis.

J. Donate-Correa et al. (8) systematize modern knowledge about the basic system of regulation of phosphorous homeostasis - FGF-23 and Klotho, and its involvement in the pathogenesis of various diseases. The possibilities of measurements of these two markers are highlighted in the assessment of the progression of chronic kidney disease, acute renal failure, secondary hyperparathyroidism, vascular dysfunction, atherosclerosis, and cardiovascular morbidity and mortality.

It is assumed that there is a possible mechanism by which the extremely high concentrations of FGF-23 are involved in the destruction of endothelial thrombomodulin. This is a potential cardiovascular risk factor in patients with CKD, especially those on HD treatment (9).

The levels of FGF-23 and the protein-bound biomarker α-Klotho showed a significant increase in patients with high PTH on HD, more pronounced in females than in the control group (Fig. 1 and 2). For patients on HD, the level of FGF-23 may increase by thousands compared to healthy subjects (10).

The comparison between the two groups is presented on the next figures:

FGF-23 itself performs by activating FGF receptors in a way, dependent on Klotho. Reducing Klotho levels in serum and urine, followed by elevations in serum concentrations of FGF-23 in the

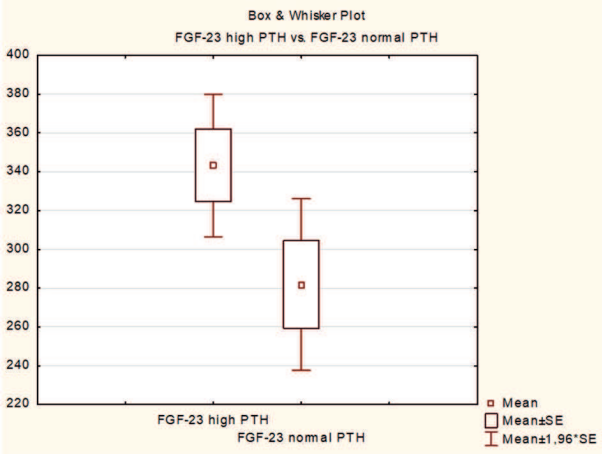

Fig. 1. Serum levels of FGF-23 - pmol/L measured in patients on HD

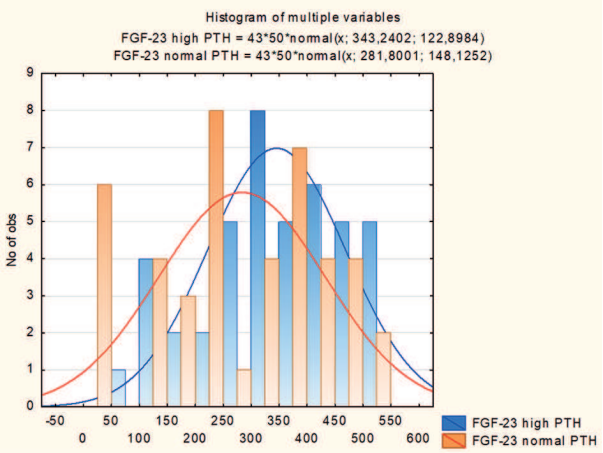

Fig. 2. Serum levels of FGF-23 in HD patients with normal and high PTH

early stages of CKD, serves as a prognostic marker for the risk of cardiovascular disease and death.

In addition, Klotho's lack is a pathogenetic factor in the progression of CKD and cardiovascular disease. FGF-23 may also contribute to the development of cardiovascular disease. Prevention of Klotho reduction, re-activation of its endogenous production, or the addition of exogenous Klotho, reduces renal fibrosis, slows the progression of CKD, improves mineral metabolism, reduces cardiomyopathy, and vascular calcification in the disease (11).

With the progression of CKD, a decrease in the serum concentration of calcium, and an increase in phosphorus, alkaline phosphatase, fibroblast growth factor-23 (FGF-23), parathyroid hormone have been established (12). It has been determined that due to treatment with calcimimetics or the administration

of low calcium dialysate, arrhythmias can occur with the appearance of prolonged QT interval (13). Vascular calcification - a leading mechanism for increased mortality, can induce endothelial dysfunction that directly stimulates vascular smooth muscle cells undergoing phenotypic changes. Hyperphosphatemia correlates with endothelial dysfunction and elevated FGF-23 (14).

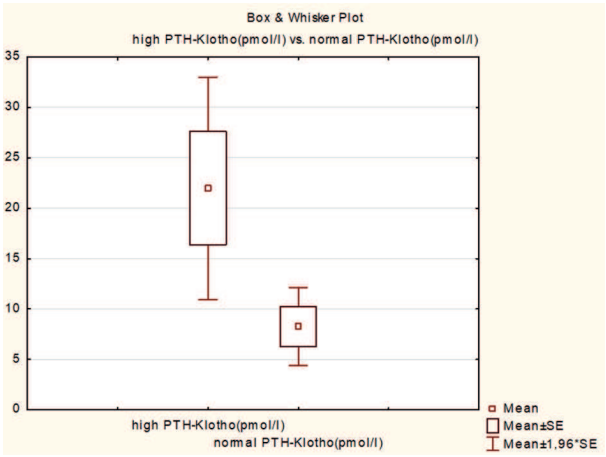

Fig. 3. Histogram of frequency distribution of the concentrations of PTH – high and normal

Studies have established a relationship between Klotho urinary levels and renal function in patients with CKD, which predict the development of renal disease in these patients. α-Klotho protects renal tubulointerstitial fibrosis induced by uretheral obstruction and urethra by the activity of multiple signaling receptors. Reduction of α-Klotho and FGF with receptor 1c - expression in parathyroid tissue is associated with secondary hyperparathyroidism. The expression of α-Klotho and FGF receptor 1c in parathyroid tissue, produced by parathyroidectomy in patients with CKD have been studied (15).

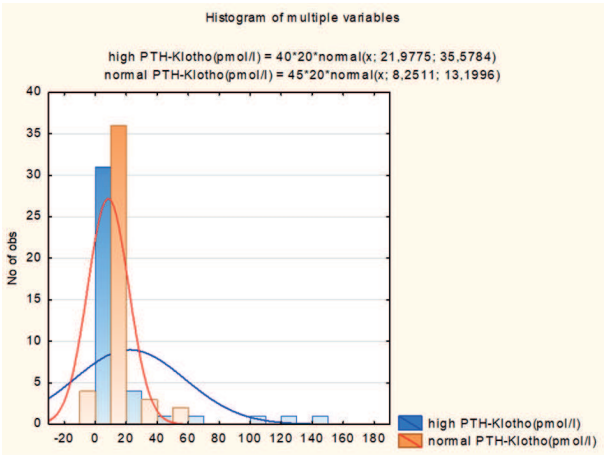

Fig. 4. Histogram of frequency distribution of the concentrations in group 1 and group 2 measured in pmol /l

Reduced Klotho expression due to renal impairment, including CKD, may increase the circulating level of FGF-23 and trigger disturbances of bone mineral metabolism in CKD (16).

CONCLUSION

Patients on dialysis treatment with high PTH values show significantly higher levels of FGF-23 in serum, especially in females, compared to patients in the control group.

Increased serum concentrations of FGF-23 at the pre-dialysis stage of CKD are a valuable prognostic marker for the risk of cardiovascular disease and mortality.

Secondary hyperparathyroidism is associated with osteitis fibrosa, early build-up of phosphorus (responsible for overgrowth of FGF-23 from bone tissue), decreased production of calcitriol from the kidneys and hypocalcemia (17). Other bone damaging factors include acidosis, chronic inflammation, food deficiency, and iatrogenic complications.

To treat hyperphosphatemia, it is recommended to administer calcium-free medications because they lead to cardiovascular calcifications, although they decrease the level of P. It is advisable to avoid oral administration of P-connecting binders containing calcium. Diet, prolonged and quality dialysis, talks and dialogues with patients and their relatives for the acceptance of selective and non-selective vitamin D receptor analogues is the key to successful treatment! Adequate therapeutic approach is needed to treat multiple CKD components in patients with bone mineral disorders. The research of modern biomarkers - FGF-23 and α-Klotho - is important for the qualitative and timely assessment of bone changes in patients with CKD.

REFERENCES

1. Kitagawa M, Sugiyama H, Morinaga H, Ogawa A, Jamanari T, Onishi A, et al. Lower levels of urinary soluble klotho significantly predict renal outcomes in patients with chronic kidney disease: a novel bio-

marker for progression chronic kidney disease. Nephrology. 2014;19:66.

2. Ivorra C, García-Vicent C, Chaves FJ, Monleón D, Morales JM, Lurbe E. Metabolomic profiling in blood from umbilical cords of low birth weight newborns. J Transl Med. 2012;10:142. doi: 10.1186/1479-5876-10-142.

3. Yonova D, Dukova P. Changes of serum bone markers in CAPD and hemodialysis patients. Hippokratia. 2007;11(4):199-201.

4. Ozdemir AA, Altay M, Celebi A, Mavis O. Literature review in the treatment of calciphylaxis: A case with uncontrolled and severe secondary hyperparathyroidism. Caspian J Intern Med. 2016; 7(1):57-60.

5. Li W, D, Zhang S. Risk factors of parathyroid dysfunction in elderly patients with chronic kidney disease undergoing hemodialysis. Adv Clin Exp Med. 2015; 24(6):1007-12. doi: 10.17219/acem/23439.

6. Fujii H, Joki N. Mineral metabolism and cardiovascular disease in CKD. Clin Exp Nephrol. 2017;21(Suppl 1):53-63. doi: 10.1007/s10157-016-1363-8.

7. Kuro-O M, Moe OW. FGF23-αKlotho as a paradigm for a kidney-bone network. Bone. 2017; 100:4-18. doi: 10.1016/j.bone.2016.11.013.

8. Donate-Correa J, Muros de Fuentes M, Mora-Fernández C, Navarro-González JF. Pathophysiological implications of fibroblast growth factor-23 and Klotho and their potential role as clinical biomarkers. Clin Chem. 2014; 60(7):933-40. doi: 10.1373/clinchem.2013.206649.

9. Tanaka K, Salunya T, Motomiya Y, Motomiya Y, Oyama Y, Yamakuchi M, et al. Decreased expression of thrombomodulin in endothelial cells by fibroblast growth factor-23/α-Klotho. Ther Apher Dial. 2017; 21(4):395-404. doi: 10.1111/1744-9987.12524.

10. Grabner A, Mazzaferro S, Cianciolo G, Krick S, Capelli I, Rotondi S, et al. Fibroblast growth factor 23: Mineral metabolism and beyond. Contrib Nephrol. 2017; 190:83-95. doi: 10.1159/000468952.

11. Palmer SC, Nistor I, Craig JC, Pellegrini F, Messa P, Tonelli M, et al. Cinacalcet in patients with chronic kidney disease: a cumulative meta-analysis of randomized controlled trials. PLoS Med. 2013;10(4):e1001436. doi: 10.1371/journal.pmed.1001436.

12. Li H, Jun-Fang Y, De-Guang W, Sheng-Xue X, Liang Y. Decreased α-Klotho and fibroblast growth factor receptor 1c expression in parathyroid is associated with secondary hyperparathyroidism in chronic kidney disease. Nephrology. 2014;19:118.

13. Cozzolino M. Which Outcome in Chronic Kidney Disease-Mineral and Bone Disorder Patients? Nephrourol Mon. 2014;6(3):e18662. doi: 10.5812/numonthly.18662.

14. Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, et al. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. Kidney Int. 2007;71(1):31-8. doi: 10.1038/sj.ki.5002009.

15. Sun Y, Shimokado A, Oikawa K, Murgaki Y. UUO-induced renal tubulointerstitial fibrosis is attenuated in Klotho-deficient mice by elevated levels of plasma vitamin D via suppressing TGF-beta signaling and renin-angiotensin system. Nephrology.2014;19:93.

16. Kimura T, Shiizaki K, Kuro-O M. Role of parathyroid hormone in Klotho-FGF23 system. Clin Calcium. 2016;26(6):859-66. doi: CliCa1606859866. (in Japanese).

17. Ballinger AE, Palmer SC, Nistor I, Craig JC, Strippoli GFM. Cochrane Database Syst Rev. 2014;(12):CD006254. doi: 10.1002/14651858.CD006254.pub2.