The aim of the study was to investigate the relationship between FGF-23, Klotho, sclerostin serum level changes and diffuse arterial stiffness, calcification intensity and myocardial remodelling in patients with different stages of Chronic Kidney Disease (CKD).

The control group consisted of 15 volunteers the same average age and sex. Serum FGF-23 levels (Human FGF-23 ELISA kit using monoclonal antibodies to the full FGF-23 molecule), Klotho (Human alpha-KI ELISA using anti-Klotho antibodies) and sclerostin (Human sclerostin ELISA kit) were applied in these patients. Blood pressure (BP) was measured to all study patients. Echocardiography was performed to patients with arterial hypertension and left ventricular mass index (LVMI) was calculated. The state of blood flow in the heart and large vessels (Doppler ultrasound Echocardiography), pulse wave velocity (Sphigmokor), calcifications presence (echocardiography, radiography of abdominal aorta by Kauppila method) and vascular wall functional ability (augmentation indices by Sphigmokor) were studied. Among 49 hypertensive patients in 27 (55.1%) from them it was able to maintain target BP-120/80-140/80 mm Hg., the remaining 22 (44.9%) patients took antihypertensive medications irregularly. At the start of screening they had been remained hypertensive (BP 150/90-165/100 mm Hg.).

Our study demonstrated that serum levels of Klotho, FGF-23, sclerostin are early markers of cardiovascular events in CKD. It was found the clear link between increased serum FGF-23 and decreased Klotho and sclerostin as increasing CKD severity, and diffuse arterial stiffness and calcification, myocardial remodelling independent of traditional risk factors. To the final determination of the sclerostin role in CKD more exactly further researches are required.

**Keywords:** Chronic kidney disease, ectopic calcification, fibroblast growth factor-23, left ventricular hypertrophy, sclerostin, soluble alpha-Klotho

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**Introduction**

Chronic renal failure (chronic kidney disease - CKD stages III-VD) spreads widely in the population (10-15% of the population), is characterized by a high rate of cardiovascular events (CVE) (Couser, Remuzzi, Mendis, Tonelli, 2011; KDIGO, 2009; Milovanova, Milovanov, Plotnikova, 2012), the risk of which, including young people, increases 100 and more times (Kuo-Cheng Lu, Chia-Chao Wu, Jen-Fen Yen et al., 2014).

In the genesis of CVE in CKD many mechanisms have a value. Among them, in recent years the dysfunction of morphogenetic proteins (FGF-23 and Klotho) plays a growing role...
role. According to Gutierrez O.M. et al. (Gutierrez, Januzzi, Isacova et al., 2009), high levels of FGF-23 correlate with an increase in the left ventricular mass index (LVMI). Klotho gene damage in experimental mice causes an ectopic calcification, pathological fractures, premature aging (Hu, Shi, Zhang et al., 2011; Kuro-o, 2009). On the other hand, the circulating form of Klotho protein can function as a humoral factor that protects the cardiovascular system (Mikolova, Milovanov, Kozlovskaya, 2013; Ming Chang Hu, Makoto Kuro-o, Orson Moe, 2013a). Overexpression of the Klotho protein provides both renal and cardiovascular protection (Ming Chang Hu et al., 2013a; 2013b). Furthermore, recently has been identified a new factor secreted by osteocytes glicoprotein sclerostin which involved in the regulation of bone formation and osteoblastogenesis (Silverman, 2010). At the same time in the literature, there is a hypothesis about its impact on the process of arteries and heart valves calcification in patients with CKD (Kuo-Cheng Lu et al., 2014).

Most studies of morphogenetic proteins were carried out on a dialysis population and in world literature to date, no conclusive data about the role of FGF-23 and Klotho in cardiorenal relationships in patients with early stages of CKD. As for sclerostin, with regard to this biomarker are only a few reports.

**Materials and methods**

65 patients with CKD 1-5D stage were included in the study: 25 - with chronic glomerulonephritis, 20 - tubulointerstitial nephritis 20 - hypertensive nephrosclerosis (33 men and 32 women, 20-65 years old, mean age at enrollment was 41±6.7 years). The control group consisted of 15 volunteers the same average age and sex. Serum FGF-23 levels (Human FGF-23 ELISA kit using monoclonal antibodies to the full FGF-23 molecule), Klotho (Human alpha-Kl ELISA using anti-Klotho antibodies) and sclerostin (Human Sclerostin ELISA kit) were applied in these patients. Blood pressure (BP) was measured in all study patients.

CKD stage was determined according to the criteria NKF KDOQI Guidelines (2002), with GFR calculated by the equation CKD-MDRD (KDIGO, 2009).

The criteria do not include patients in the study were the presence of nephritis activity, serious infectious complications at the study period. All patients were performed screening clinical examination, including PTH, calcium and phosphorus serum levels determination.

Among the 65 patients included in the study, at the time of blood sampling, 21 patients (32.3%) had normal blood pressure (120 / 80-140 / 80 mm Hg. V.), and 49 (75.3%) patients had arterial hypertension of different severity.

Among 49 hypertensive patients in 29 (59.1%) from them it was able to maintain target BP-130/80-140/80 mm Hg., the remaining 20 (40.7%) patients took antihypertensive medications irregularly. At the start of screening they remained hypertensive (BP 150/90-165/100 mm Hg).

Echocardiography was performed according to a standard protocol (Gosse et al 1990, Devereux 1990) (Devereux, 1990) and left ventricular mass index (LVMI) was calculated. The state of blood flow in the heart and large vessels (Doppler ultrasound Echocardiography), pulse wave velocity (Sphigmokor), calcifications presence (echocardiography, radiography of abdominal aorta by Kauppila method) and vascular wall functional ability (augmentation indices by Sphigmokor) also were studied.

All participants gave written informed consent.

For statistical analysis it was used SPSS software for Windows 17 with performance of correlation analysis, regression analysis with charting. Correlations between two variables were examined by linear regression analysis, and Pearson correlation coefficient (r) was expressed. Two-sided P < 0.05 was considered to indicate statistical significance.
Results

Strong direct correlation ($r=0.731$, $p<0.01$) was established between stage of CKD by MDRD and serum FGF-23 levels (Figure 1a), inverse correlations ($r=-0.489$, $p<0.01$) and ($r=-0.510$, $p<0.01$) were established between stage of CKD and Klotho (Figure 1b) and stage of CKD and sclerostin (Figure 1c) levels respectively.

**Figure 1A. Change in FGF-23 serum levels depending on CKD stage**

**Figure 1B. Change in Klotho serum levels depending on CKD stage**
When comparing serum FGF-23, Klotho and sclerostin serum levels in patients with different CKD stages was found FGF-23 levels increasing and Klotho and sclerostin levels decreasing as the deterioration of GFR ahead of serum phosphorus (Figure 2a) and PTH levels (Figure 2b) elevating, starting at CKD III a stage, whereas hyperphosphatemia and increased PTH levels were started in CKD IV-V stage.
We assessed the serum morphogenetic proteins changes depending on BP levels. The degree of increasing blood pressure correlated positively with FGF-23 serum levels ($r=0.452;\ p<0.01$) (Figure 3a) and inversely with Klotho levels (Figure 3b) ($r=-0.687;\ p<0.01$). Significant correlation of sclerostin levels with the degree of hypertension has not been received.
We also established the strong straight relationship of FGF-23 serum levels (Figure 4a) \( (r=0.492, p<0.01) \) and the reverse relationship of serum Klotho levels (Figure 4b) \( (r=-0.537; p<0.01) \) and sclerostin serum levels (Figure 4c) \( (r=-0.541, p<0.05) \) respectively with time of pulse valve reflection (Sphigmokor).
In addition, it was found the feedback between enhanced FGF-23 levels with increased left ventricular mass (LVMM) \( r=0.452; p<0.05 \).

In hypertensive patients \((n=20)\) this connection was extremely expressed \( r=0.850; p<0.05 \) (Figure 5).
In studied patients reduced serum Klotho and sclerostin levels have been clearly associated with a higher frequency of stiffness and calcificat identification in the heart valves (Echocardiography) (mitral valve $r=-0.492$, $p<0.01$ and $r=-0.487$, $p<0.01$, respectively) (Figure 6a) (aortal valve $r=-0.543$ $p<0.01$ and $r=-0.457$, $p<0.01$) (Figure 6b) and large arteries (abdominal aorta) (Klo-to $r=-0.525$; $p<0.01$) (Figure 7).
Reduced serum Klotho and sclerostin levels have been also associated with a concentric remodeling of the myocardium ($r=-0.445$ $p<0.01$ and $r=-0.567$, $p<0.01$ respectively) (Figure 8).
Discussion

Our results confirm experimentally established fact that morphogenetic proteins - FGF-23, Klotho and glycoprotein sclerosstin are earlier than PTH and phosphorus, markers of CKD progression and associated with cardiovascular complications (Kuo-Cheng Lu et al., 2014; Gutierrez et al., 2009; Kuro-o, 2009). Their changes begin early - stage IIIa of CKD - increase as worsening renal failure associated with progression of extra bone calcification and remodeling of heart and vessels.

As studying of the calcification mechanisms more and more evidence appear that calcification of the heart and blood vessels is not a passive process of calcium and phosphorus precipitation from the circulation. It is shown that under the influence of trigger factors (phosphorus?) in vascular smooth muscle cells(VSMC) decreases the secretion of annexin II, increased production of reactive oxygen species (ROS), decreased angiogenesis, activated Wnt / beta-catenin signaling pathway via translocation of beta-core catenin in VSMC. As a result, VSMC lose their ability to produce smooth muscle, and begin to express proteins osteoblastogenesis genes, e.g., Runx2 / Cbf1. Runx2 is a major transcription factor forking osteogenic / chondrogenic differentiation of VSMC, which leads them to the formation of vesicles, containing apatite and calcified collagen fibrils. These vesicles are initial regions containing the nucleus of the future vascular wall calcification. Thus, Wnt signaling pathway not only plays a major role in bone metabolism, but also involved in processes media calcification of the arteries and heart valves (Kuo-Cheng Lu et al., 2014; Zhu, Mackenzie, Millán, Farquharson, MacRae, 2011).

It is now established that sclerostin (glycoprotein secreted osteocytes) is a blocker of WNT signaling pathway and may be an important regulator in the development of mineralization and calcification of blood vessels and the heart together with the deleterious effects on bone metabolism (Kuo-Cheng Lu et al., 2014; Román-García, Carrillo-López, Fernández-Martín et al., 2010.). Our data support an association of low serum sclerostin levels with a frequency of calcification of the heart and blood vessels. However, more studies are demanded for better understanding its role in CKD.
Elevated serum concentrations of FGF-23 and decreased expression of Klotho and sclerostin in CKD occurs in parallel of drooping in GFR, reaching maximal changes in patients receiving dialysis. We found a strong relationship between increased serum levels of FGF-23 - direct and decrease the concentration of Klotho and sclerostin - reverse, and a high risk of cardiovascular complications (CVC) in patients with CKD.

Under the active nephron mass reduction conditions and reduction of existing nephrons receptor to FGF-23 (FGFRI) in kidney with an increase of FGF-23 levels in the serum its effects can spread to any other organs expressing FGFRI (for example - on the myocardium). In a number of fairly large observational studies have directly been shown that the increase in FGF-23 serum levels results in remodeling of the heart and blood vessels (Isha Agarwal, Noriko Ide, Joachim H. Ix et al., 2014; Julia Scialla, Huiliang Xie, Mahboob Rahman et al., 2014) regardless of the serum levels of phosphorus, which was within the normal range in most patients (Jean, Terrat, Vanel et al., 2009).

According to the literature, in transgenic mice increased expression of Klotho in CKD was combined with adequate phosphaturia, better functional ability of the kidneys and significantly lower degree of calcification compared with wild-type mice with CKD and reduced production of Klotho (Ming Chang Hu et al., 2013a). Moreover, the favorable effects on vascular calcification of Klotho were expressed to a greater extent than its effects on renal function and phosphaturia, which is seen as an association with a direct effect of Klotho to the blood vessels.

Do we observed the patients with hypertension Klotho deficiency correlated with a higher degree of it, the presence of calcifications in the heart and blood vessels and increase the rigidity of the peripheral arteries.

Conclusion

Our study had demonstrated that serum levels of Klotho, FGF-23, sclerostin are early markers of cardiovascular events in CKD. It was found the clear link between increased serum FGF-23, decreased Klotho and sclerostin as increasing CKD severity, and diffuse arterial stiffness and calcification, myocardial remodeling independent of traditional risk factors. To the final determination of the sclerostin role in CKD more exactly further researches are required.

References