Int Urol Nephrol (2017) 49:1809–1814
DOI 10.1007/s11255-017-1646-3

NEPHROLOGY - ORIGINAL PAPER

Klotho levels: association with insulin resistance and albumin-to-creatinine ratio in type 2 diabetic patients

Ana Paula Silva[1,2] · Filipa Mendes[1] · Luísa Pereira[1] · André Fragoso[1] · Rui Baptista Gonçalves[2] · Nélio Santos[3] · Fátima Rato[3] · Pedro Leão Neves[1,2]

Received: 2 April 2017 / Accepted: 26 June 2017 / Published online: 4 July 2017

Abstract
Purpose The present study aimed at evaluating the relationship between Klotho levels and insulin resistance and albumin-to-creatinine ratio (ACR) in type 2 diabetic patients with CKD.
Methods We conducted an observational, cross-sectional study in our outpatient diabetic nephropathy clinic from 2014 to 2016, enrolling a total of 107 type 2 diabetic patients with stage 2–3 CKD, with a mean age of 59 years. Several clinical and laboratorial parameters were evaluated, including those related to mineral and carbohydrate metabolism.
Results The mean eGFR at baseline was 53.2 mL/min, and the mean levels of ACR and Klotho were 181.9 µg/mg and 331.1 pg/m, respectively. In the simple linear regression model, Klotho levels were correlated with age, phosphorus, PTH, ACR, HOMA, IL-6, FGF-23, OxLDL, eGFR and vitamin D levels. Applying a multivariate linear regression model, only the ACR, HOMA-IR, FGF-23 and vitamin D independently influenced the Klotho levels. In the generalized linear model, only the Klotho groups were statistically significant as independent variable ($p = 0.007$). The results show that the group 1 (<268) compared with group 3 (>440) had higher odds in the higher ACR (≥181), ORa = 3.429, $p = 0.014$. There were no statistically significant differences between Klotho groups 2 and 3, and the HOMA-IR obtained showed that group 1 (<268) had greater odds of HOMA-IR ≥2 when compared with group 3 (>440), ORa = 21.59, $p = 0.017$.
Conclusions Our results showed that Klotho levels are influenced by FGF23, vitamin D and insulin resistance. This suggests that Klotho levels might be affected by renal function as well as having a relevant role on insulin metabolism and ACR homeostasis.

Keywords Klotho · Chronic kidney disease · Diabetes · Mineral metabolism

✉ Ana Paula Silva
anapassionara@gmail.com

[1] Department of Nephrology, Centro Hospitalar do Algarve, Faro, Portugal

[2] Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal

[3] Pathology Clinic, Centro Hospitalar do Algarve, Faro, Portugal

Introduction

Klotho is a protein expressed in several organs and tissues, with greater predominance in the kidney and brain. It has been implicated in multiple organic processes, being able to regulate growth factors signaling pathways, ion channels and transporters [1].

Klotho plays a particularly relevant role within the bone-kidney endocrine axis. In association FGF-23, it mediates the phosphate excretion and homeostasis through the inhibition of $1.25(OH)_2$ vitamin D3 synthesis and the induction of phosphaturia [2].

Recently, increasing evidence links Klotho levels with chronic kidney disease (CKD), as basic and clinical research have shown the link between Klotho deficiency and CKD [3–5] stages. Moreover, Klotho also seems to play a renoprotective role through its anti-oxidation properties: protection of vasculature, promotion of vascularization and inhibition of fibrinogenesis [6]. Therefore, it appears reasonable to consider Klotho as a potential surrogate

biomarker and target for early intervention and treatment of CKD.

Despite this, the association between Klotho and CKD is still far from being fully understood, as some studies have arrived at divergent conclusions [7, 8]. The discrepancy of results may well be explained by the multifactorial pathophysiology of CKD, especially in patients with comorbidities, such as diabetics.

In this group, some studies argued that Klotho levels may be able to mediate insulin metabolism through the inhibition of intracellular insulin and IGF1 signaling [9], as well as to enhance glucose-induced insulin secretion [10].

Furthermore, different studies have speculated that Klotho levels might also be correlated with the albumin-to-creatinine ratio (ACR) both by direct [11, 12] and indirect mechanisms, with low Klotho levels being associated with proteinuria.

In the present study, our objective was to evaluate the relationship between Klotho levels and insulin resistance and ACR. We intended to understand Klotho's role in insulin resistance, as well as to examine whether it played a renoprotective role in type 2 diabetics with chronic renal disease.

Methods

Subjects

An observational, cross-sectional study was conducted in our outpatient diabetic nephropathy clinic from 2014 to 2016, enrolling a total of 107 type 2 diabetic patients with stage 2–3 CKD. The diabetes classification was based on the American Diabetes Association guidelines [13].

The exclusion criteria were: age > 75 years, previous CVD (defined as history of one or more of the following: non-fatal myocardial infarction, (stable or unstable) angina pectoris, stroke or transient ischemic attacks, peripheral vascular disease or congestive heart failure); uncontrolled hypertension (BP $\geq$ 140/90 mmHg), albumin-to-creatinine ratio (ACR) >500, estimated glomerular filtration rate (eGFR) $\leq$29 or >90 mL/min, type 1 diabetes, known neoplastic or infectious diseases, non-diabetic renal disease (patients without previous history of diabetes, with diagnosis of glomerulopathies associated with other pathologies like systemic diseases, IgA nephropathy, kidney disease of unknown ethology, chronic interstitial nephritis, vasculitis, component complement 3 pathologies or renal hereditary diseases. Patients with parathyroid hormone (PTH) $\geq$350 pg/mL were excluded to avoid bias from anti-hyperparathyroidism medication that can affect Klotho and FGF-23 levels. The same happened with patients with phosphorus >5.5 and the phosphorus-chelating agents that can affect the Klotho-FGF-23 axis. Patients undergoing therapy with vitamin D and vitamin D receptor activators and phosphate binders were also excluded.

Blood measurements

Fasting samples were drawn from all subjects, and plasma was frozen at −80 °C in order to measure eGFR, phosphorus (P), calcium (Ca), PTH, ACR, insulin resistance degree, interleukin-6 (IL-6), fibroblast growth factor-23 (FGF 23), $1.25(OH)_2D3$ (vitamin D), oxidized low density lipoprotein (oxLDL) and soluble α-Klotho (Klotho). Serum levels of FGF-23 were quantified using an enzyme-linked immunosorbent assay, *Human FGF-23 (C-Term)* ELISA kit (Cat. #60-6100 Immunotopics Inc, San Clemente, CA, USA). Serum levels of vitamin D were determined with the help of a radioimmunoassay (IDS, Boldon, UK). Phosphorus and calcium were assayed by the ARCHITECTc Systems and the AEROSET System (Abbott Diagnostics Division, Abbott Laboratories Abbott Park, IL). IL-6 and oxLDL determination was done with a sandwich enzyme-linked immunoassay (ELISA) kit (eBioscience, San Diego, California). PTH levels were measured using an electrochemiluminescent immunoassay (ECLIA). PTH concentrations were measured on an Immulite 2000 Intact PTH assay (Cat. #L2KPP2, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Soluble α-Klotho serum levels were determined by enzyme-linked immunosorbent assay using the "Human soluble α-Klotho ELISA kit" (Code no-27998, IBL—Immuno-Biological Laboratories Co., Ltd, Gunma, Japan), according to manufacturer's instructions and adapted to Triturusmicroplate automatic system (Grifols S.A., Barcelona, Spain). The degree of insulin resistance was estimated using the homeostasis model assessment (HOMA-IR) described by Matthews et al. [14]. Serum creatinine was assayed by the enzymatic method, using the ARCHITECT® device (Abbott Diagnostics Division, Abbott Laboratories Abbott Park, IL). We estimated the GFR according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equation [15].

Statistical analysis

Descriptive quantitative data were presented as mean and standard deviation. Simple linear regressions were used to investigate possible correlations between these variables and Klotho. Only the variables with a statistically significant relationship were introduced in a multiple regression model. The generalized linear model (GLM) for binary dependent variables was used (binomial distribution) with a logistic link function. The exponentials of the model parameters were the adjusted odds ratio (ORa) to other variables of the model. The test was used to determine whether

Klotho was an important predictive factor in the determination of the albumin-to-creatinine event and the resistance to insulin. Prior to conducting the GLM, the 25th, 50th and 75th percentiles of serum Klotho level in the current study population were determined. The subjects were categorized as follows: group 1 (Klotho < 268), group 2 (Klotho: 268–440) and group 3 (Klotho > 440).

The null hypothesis was rejected below the level of 5%. Statistical analysis was performed using SPSS (version 17.0; SPSS, Chicago, IL).

Institutional Ethics Committee approval (reference 207/2010) was obtained for this study.

Results

One hundred and seven (107) consenting patients with stage 2–3 CKD were included in the study after confirming they did not meet any of the exclusion criteria.

The mean age was 59 ± 8.6 [range 41–72] years, and the mean Klotho level was 331.10 ± 171.06 [range 70–663 pg/mL]. The demographic and clinical parameters are presented in Table 1.

Simple linear regression (Table 2) demonstrated that Klotho levels were inversely correlated with age ($r = -0.232$, $p = 0.016$), phosphorus ($r = -0.381$, $p < 0.001$), PTH ($r = -0.606$, $p < 0.001$), ACR ($r = -0.336$, $p < 0.001$), HOMA-IR ($r = -0.482$, $p < 0.001$), IL-6 ($r = -0.571$, $p < 0.001$), FGF-23 ($r = -0.695$, $p < 0.001$), OxLDL ($r = -0.598$, $p < 0.001$) and directly correlated with eGFR ($r = 0.234$, $p = 0.021$) and vitamin D ($r = 0.661$, $p < 0.001$) levels.

Applying the multivariate linear regression (Table 3), only the ACR ($r = -0.636$ $p = 0.036$), HOMA ($r = -0.322$, $p = 0.018$), FGF-23 ($r = -0.668$ $p < 0.001$) and vitamin D ($r = 8.465$ $p = 0.010$) independently influenced Klotho levels.

In the generalized linear model (GLM), only the Klotho groups were statistically significant as independent variable ($p = 0.007$). The results show that group 1 (<268) compared to group 3 (>440) had higher odds in the higher ACR (≥181), ORa = 3.429, $p = 0.014$. Group 2 had no statistical significant difference compared to group 3. The optimized model as function of Klotho groups, clearance values, age, phosphorus, PTH, IL6, vitamin D, OxLDL and FGF-23 allowed for a final and statistical significant model ($p < 0.001$) which included Klotho groups and vitamin D variables. The area under the ROC curve for this optimized model was 0.772

Table 1 Patients demographic and clinical characteristics at baseline

Characteristics	Values
Number of patients (n)	107
Gender (f/m)	40/67
Age (years)	59.00 ± 8.57
Hb (g/dL)	12.97 ± 1.83
Albumin (g/dL)	4.27 ± 0.48
eGFR (mL/min)	53.20 ± 10.15
ACR (μg/mg)	181.89 ± 123.83
Phosphorus (mg/dL)	3.99 ± 0.85
PTH (pg/mL)	113.11 ± 74.65
Calcium (mg/dL)	9.43 ± 0.92
FGF-23 (RU/mL)	135.04 ± 35.23
[1.25(OH)$_2$D3] (pg/mL)	21.18 ± 7.37
IL-6 (pg/mL)	5.71 ± 3.80
OxLDL (U/L)	39.91 ± 19.55
α-Klotho (pg/mL)	331.10 ± 171.06
HOMA-IR	1.84 ± 1.67
HbA1c	7.8 ± 2.0

Values are presented as mean ± standard deviation

Table 2 Simple linear regression analysis between Klotho and other parameters in diabetic patients with CKD

Variable	*R*	*p* value
Age	−0.232	0.016
eGFR	0.234	0.021
Phosphorus	−0.381	<0.001
Calcium	0.090	0.354
PTH	−0.606	<0.001
ACR	−0.336	<0.001
HOMA-IR	−0.482	<0.001
IL-6	−0.571	<0.001
FGF-23	−0.695	<0.001
Vit.D	0.661	<0.001
OxLDL	−0.598	<0.001

Table 3 Multivariate linear regression analysis between Klotho and other parameters in diabetic patients with CKD

Variable	Coefficient	SE	*p* value
Age	0.222	1.599	0.890
eGFR	0.090	0.626	0.886
Phosphorus	11.909	21.277	0.577
Calcium	2.424	10.701	0.821
PTH	−0.291	0.253	0.252
ACR	−0.636	0.105	0.036
HOMA-IR	−0.322	0.588	0.018
IL-6	−7.889	6.098	0.199
FGF-23	−0.668	0.135	<0.001
Vit.D	8.465	3.225	0.010
OxLDL	1.856	1.223	0.132

($p < 0.001$), revealing a sound discriminant capacity of the model. The estimates obtained showed that group 1 (<268) compared with group 3 (>440) had greater odds of ACR ≥181 (ORa = 1.324, $p = 0.024$) and that higher levels of vitamin D were statistically associated with lower values of ACR (ORa = 0.808, $p < 0.001$) (Table 4).

No statistical significant difference was found between groups 2 and 3, but the HOMA-IR obtained showed that group 1 (<268) had greater odds of HOMA-IR ≥2 (ORa = 21.59, $p = 0.017$) when compared with group 3 (>440). The area under the ROC curve for this optimized model was 0.978 ($p < 0.001$), revealing an excellent discriminant capacity of the model (Table 5).

Discussion

In the last few years, Klotho has become an ineluctable topic of discussion among nephrologists, due to its known contribution to mineral metabolism homeostasis in general and phosphate handling in particular. For this reason, Klotho has been recurrently seen as a promising target for the management and treatment of patients with CKD. Nevertheless, contradictory recent evidence has raised questions on whether Klotho actually plays any active role in CKD.

CKD is a well-known multifactorial disease, with several contributing factors and associated triggering agents. Most of these seem to be linked in a well-arranged web, and a single deregulation is able to affect the whole chain, producing different pathological outcomes. The addition of other comorbidities such as diabetes often leads to an extreme complex puzzle of interconnections which may be difficult to understand.

The current study investigated the correlations between serum Klotho levels and ACR and insulin resistance, in type 2 diabetic patients with stage 2–3 CKD.

Results showed that Klotho levels were correlated with FGF23, vitamin D and insulin resistance, suggesting that Klotho levels might be affected by renal function. This seems to be in accordance with most of the studies that describe CKD as a state of FGF-23 resistance caused by the deficiency of Klotho [16]. Nevertheless, this is still a debatable subject, as conflicting results have reported that Klotho levels were significantly correlated with age, but not with eGFR or other parameters of mineral metabolism in patients with CKD [8].

Table 4 GLM for the albumin-to-creatinine ratio (≥181)

	Initial model			Optimized model		
	OR_a	95% CI for OR_a	*p* value	OR_a	95% CI for OR_a	*p* value
Klotho groups						
<268	3.429	1.288–9.125	0.014	1.324	1.061–1.721	0.024
268–440	0.839	0.302–2.328	0.736	0.586	0.182–1.886	0.370
>440	Ref.			Ref.		
Vitamin D	–	–	–	0.808	0.725–0.901	<0.001
p value (model)	0.007			<0.001		
Area under ROC (*p* value)	–			0.772 ($p < 0.001$)		

GLM generalized linear model, *ORa* adjusted odds ratio, *95% CI for OR* 95% confidence interval for the odds ratio, *Ref* category versus the one is making comparisons

Table 5 GLM for the HOMA-IR (≥2)

	Initial model			Optimized model		
	OR_a	95% CI for OR_a	*p* value	OR_a	95% CI for OR_a	*p* value
Klotho groups						
<268	30.000	8.256–109.006	<0.001	21.590	1.733–268.903	0.017
268–440	2.640	0.809–8.616	0.108	7.264	0.847–62.332	0.071
>440	Ref.			Ref.		
p value (model)	<0.001			<0.001		
Area under ROC (*p* value)				0.978 ($p < 0.001$)		

GLM generalized linear model, *ORa* adjusted odds ratio, *95% CI for OR* 95% confidence interval for the odds ratio, *Ref* category versus the one is making comparisons

In this study, Klotho levels were associated with insulin resistance inasmuch as being a predictive factor for insulin resistance, as indicated by the optimized generalized model (Table 5). The association between Klotho levels and insulin resistance also seems to be in accordance with other studies [9, 10]. These studies reported that Klotho levels can mediate insulin metabolism through the inhibition of tyrosine phosphorylation of insulin and IGF1 receptors, as well as enhancing glucose-induced insulin secretion via upregulating membrane retention of TRPV2. If the apparent Klotho deficiency seen in CKD stages is taken into consideration, we might hypothesize that low Klotho levels could result in excessive insulin release, leading to a state of insulin resistance.

When our variables were transferred to the multiple regression model, the link between Klotho and eGFR was lost. Klotho was mainly associated with ACR. In the generalized linear model, used to investigate the relationship of possible predictors, only Klotho was a predictive factor in the determination of the album-to-creatinine event (Table 4). This association seems, once again, to be in line with other recent studies. These have hypothesized and investigated direct and indirect mechanisms that could possibly explain how these two variables influence each other. On a direct level, it has been argued that interstitial inflammation induced by proteinuria may downregulate Klotho expression. According to Moreno et al. [11], the release of inflammatory cytokines such as tumor necrosis factor (TNF)-like weak inducer of apoptosis' (TWEAK) and TNF-α was responsible for the downregulation of Klotho expression through a nuclear factor kappa-B-dependent mechanism. This seems to be a valid hypothesis to support the correlation between Klotho levels and ACR found in our study. The observations from Moreno et al. were supported by another study that reported an increased Klotho circulating level in type 2 diabetic patients whose proteinuria was ameliorated with losartan [12]. According to the authors, the use of renin-angiotensin system antagonists with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers in patients with diabetic nephropathy was able to reverse Klotho's downregulation induced by angiotensin II, therefore retarding disease progression.

Another direct mechanism thought to be part of the relationship Klotho/ACR is the one involving the vascular endothelial growth factor A (VEGF-A). It is believed that VEGF-A plays a pathogenic role in diabetic nephropathy, particularly on angiogenesis and vascular permeability. In their study on diabetic patients, Kacso and colleagues [17] observed a consistent positive relationship between soluble Klotho and VEGF-A (both factors having low levels in the presence of microalbuminuria). It is still to be understood whether they both respond to the same pathogenic trigger or whether it is a reactive action to the other. Nevertheless, we may hypothesize that the downregulation Klotho/VEGF-A can lead to ACR worsening through their impact on endothelial dysfunction.

In addition to these mechanisms, Klotho has also an endogenous anti-fibrotic function via antagonism of Wnt/β-catenin signaling, which promotes fibrinogenesis. Therefore, it is reasonable to argue that a loss of Klotho may be associated with the progression of diabetic nephropathy and ACR worsening by accelerated fibrinogenesis [18, 19].

Some studies have also speculated that the correlation between Klotho and ACR may be due to indirect mechanisms as well. Within the Klotho/FGF-23/vitamin D axis, it is already known that low Klotho levels are associated with increased FGF-23 and decreased vitamin D levels. FGF-23 is then able to indirectly increase proteinuria by diminishing calcitriol synthesis [20] and inducing endothelium dysfunction [21]. It is also negatively correlated with vitamin D levels in diabetic nephropathy patients with microalbuminuria [22, 23]. Thus, it is not unreasonable to hypothesize that Klotho and ACR levels might be correlated through indirect, albeit unclear but possibly FGF-23-mediated, mechanisms.

Despite some limitations, namely the small size of the sample and the limited statistical power of the applied, this study offers an added value by generating hypotheses. Notwithstanding this, further studies are needed to determine the degree of correlation between plasma levels of soluble Klotho and ACR.

Conclusions

The current study found that Klotho levels were influenced by FGF-23, vitamin D and insulin resistance, variables that are affected by the renal function. Despite this, the eGFR lost its relationship with Klotho in the multiple regression model. The generalized linear model revealed that Klotho was the sole predictive factor in the determination of the album-to-creatinine event and insulin resistance. Additional studies are required in clarifying the meaning of the relationship between Klotho and ACR, and the links between Klotho and insulin resistance also demands further analysis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All performed procedures were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Izquierdo MC, Perez-Gomez MV, Sanchez-Niño MD, Sanz AB, Ruiz-Andres O, Poveda J et al (2012) Klotho, phosphate and inflammation/ageing in chronic kidney disease. Nephrol Dial Transplant 27(Supple 4):iv6–iv10
2. Nakatani T, Sarraj B, Ohnishi M, Densmore MJ, Taguchi T, Goetz R et al (2009) In vivo genetic evidence for Klotho-dependent, fibroblast growth factor 23 (Fgf23)—mediated regulation of systemic phosphate homeostasis. FASEB J 23:433–441
3. Bernheim J, Benchetrit S (2011) The potential roles of FGF-23 and Klotho in the prognosis of renal and cardiovascular diseases. Nephrol Dial Transplant 0:1–6
4. Barker SL, Pastor J, Carranza D, Quiñones H, Griffith C, Goetz R et al (2014) The demonstration of αKlotho deficiency in human chronic kidney disease with a novel synthetic antibody. Nephrol Dial Transplant 0:1–11
5. Koh N, Fujimori T, Nishiguchi S, Tamori A, Shiomi Nakatani T et al (2001) Severely reduced production of klotho in human chronic renal failure kidney. Biochem Biophys Res Commun 280:1015–1020
6. Hu MC, Kuro-o M, Moe OW (2012) The emerging role of Klotho in clinical nephrology. Nephrol Dial Transplant 27:2650–2657
7. Hu MC, Kuro-o M, Moe OW (2012) Secreted klotho and chronic kidney disease. Adv Exp Med Biol 728:126–157
8. Seiler S, Wen M, Roth HJ, Fehrenz M, Flügge F, Herath E et al (2013) Plasma Klotho is not related to kidney function and does not predict adverse outcome in patients with chronic kidney disease. Kidney Int 83:121–128
9. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P et al (2005) Suppression of aging in mice by the hormone Klotho. Science 309:1829–1833
10. Lin Y, Sun Z (2012) Antiaging gene Klotho Enhances glucose-induced insulin secretion by up-regulating plasma membrane levels of TRPV2 in MIN6 β-cells. Endocrinology 153:3029–3039
11. Moreno JA, Izquierdo MC, Sanchez-Niño MD, Suárez-Alvarez B, Lopez-Larrea C, Jakubowski A et al (2011) The inflammatory cytokines TWEAK and TNFα reduce renal klotho expression through NFkB. J Am Soc Nephrol 22:1315–1325
12. Lim SC, Liu JJ, Subramaniam T, Sum CF (2014) Elevated circulating alpha-klotho by angiotensin II receptor blocker losartan is associated with reduction of albuminuria in type 2 diabetic patients. J Renin Angiotensin Aldosterone Syst 15:487–490
13. American Diabetes A (2010) Diagnosis and classification of diabetes mellitus. Diabetes Care 33:S62–S69
14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
15. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J (2009) CKD-EPI (chronic kidney disease epidemiology collaboration). A new equation to estimate glomerular filltration rate. Ann Intern Med 150:604–612
16. Sawires HK, Essam RM, Morgan MF, Mahmoud RA (2015) Serum klotho: relation to fibroblast growth factor-23 and other regulators of phosphate metabolism in children with chronic kidney disease. Nephron 129:293–299
17. Kacso IM, Bondor CI, Kacso G (2012) Soluble serum Klotho in diabetic nephropathy: relationship to VEGF-A. Clin Biochem 45:1415–1420
18. Haruna Y, Kashihara N, Satoh M, Tomita N, Namikoshi T, Sasaki T et al (2007) Amelioration of progressive renal injury by genetic manipulation of Klotho gene. Proc Natl Acad Sci USA 104:2331–2336
19. Lindberg K, Amin R, Moe OW, Hu MC, Erben RG, Östman Wernerson A et al (2014) The kidney is the principal organ mediating klotho effects. J Am Soc Nephrol 25:2169–2175
20. Kocełak P, Olszanecka-Glinianowicz M, Chudek J (2012) Fibroblast growth factor 23—structure, function and role in kidney diseases. Adv Clin Exp Med 21:391–401
21. Yilmaz MI, Sonmez A, Saglam M, Yaman H, Kilic S, Demirkaya E et al (2010) FGF-23 and vascular dysfunction in patients with stage 3 and 4 chronic kidney disease. Kidney Int 78:679–685
22. Silva AP, Fragoso A, Neves PL (2014) Relationship of vitamin d with diabetes mellitus and diabetic nephropathy. Port J Nephrol Hypertens 28:108–118
23. de Zeew D, Agarwal R, AmdahM Audhya P, Coyne D, Garimella T et al (2010) Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study). A randomised controlled trial. Lancet 376:1543–1551