Sclerostin, TNF-alpha and Interleukin-18 Correlate and are Together with Klotho Related to Other Growth Factors and Cytokines in Haemodialysis Patients

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Sclerostin, TNF-alpha and Interleukin-18 Correlate and are Together with Klotho Related to Other Growth Factors and Cytokines in Haemodialysis Patients

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Abstract

Patients with chronic renal failure are known to have renal osteodystrophy (bone disease) and increased calcification of vessels. A new marker of bone disease, sclerostin, the two pro-inflammatory cytokines tumour necrosis factor-alpha (TNF-alpha) and interleukin-18 (IL-18), and the fibroblast growth factor-23 (FGF-23) receptor-associated marker Klotho were tested in 84 haemodialysis (HD) patients and in healthy controls. The patients had significantly higher levels of the three former markers than of the controls while Klotho was significantly higher in the controls. Low level, but significant, correlations were observed in the patient group when the levels of these four markers were compared to each other and to those of 5 cytokines and growth factors tested earlier; high-sensitive CRP (hsCRP), interleukin-6 (IL-6), hepatocyte growth factor (HGF), fibroblast growth factor-23 (FGF-23) and soluble urokinase plasminogen activator (suPAR). Ln sclerostin correlated positively to Ln hsTNF-alpha, Ln HGF and Ln suPAR. Ln hsTNF-alpha correlated positively to Ln sclerostin, Ln hsCRP, Ln IL-6, Ln FGF-23, Ln suPAR and Ln IL-18. Ln IL-18 correlated positively to Ln suPAR and Ln TNF-alpha. Ln Klotho correlated negatively to Ln hsCRP but did not correlate to Ln FGF-23. The markers studied here may be involved in the calcification of vessels seen in HD patients due to a combination of inflammation and bone disease. The mechanisms are still not fully known but may be of importance for future therapeutic possibilities in this group of patients.

Introduction

Renal osteodystrophy is a term for several forms of bone disease as osteitis fibrosa, osteomalacia, and adynamic bone disease, which may occur in patients with chronic renal failure [1]. A new marker, sclerostin, a protein encoded by the SOST gene, is a secreted glycoprotein. Sclerostin has been shown to increase with declining renal function and is elevated in haemodialysis (HD) patients [2, 3]. It has been shown to be part of the parathyroid hormone (PTH)–calcium–phosphate–vitamin D axis [4, 5] and has been suggested to be a marker of decreased osteoblastic activity and thus a marker of the bone disease, which occurs in HD patients [5, 6]. This marker may also be associated with the inflammatory vessel disease and the calcification of vessels, which occurs in this category of patients [6, 7]. Sclerostin is produced by osteocytes similar to fibroblast growth factor 23 (FGF-23) and might upregulate the FGF-23 production by the osteocyte. FGF-23 may in turn act as a mineralization

inhibitor [8]. FGF-23 and phosphorous levels are considered to be of importance in the vascular calcification process [4, 9]. The therapy given to HD patients against secondary hyperparathyroidism is also known to affect markers of bone mineral disease [10].

TNF-alpha is one of the most potent pro-inflammatory cytokines and is known to increase in the MIA syndrome (malnutrition, inflammation, and vascular calcifications) in HD patients [11]. A role for TNF-alpha and other pro-inflammatory cytokines to induce expression of sclerostin in osteoblasts has been suggested by an *in vitro* study [12]. In an earlier study, we found the pro-inflammatory cytokine interleukin-18 (IL-18) to be increased in patients with ANCA-associated vasculitis as well as in HD patients [13].

A recent study of HD patients performed in our region suggested that unknown factors affect the outcome in this category of patients [14]. In a previous study, we compared the levels of inflammatory markers and growth factors in 84 haemodialysis (HD) patients with those in 68 healthy

controls. We found elevated levels of high-sensitive CRP (hsCRP), hepatocyte growth factor (HGF), fibroblast growth factor-23 (FGF-23), and soluble plasminogen activator receptor (suPAR) in patients compared with controls even after age and sex correction. We also found correlations between several of these markers in HD patients, but only occasionally in the controls [15].

Based on this observation, we wanted to increase the number of inflammatory markers and growth factors studied in [15] and performed an extended study with four additional markers; the bone disease-associated marker sclerostin, the two pro-inflammatory cytokines TNF-alpha and IL-18, and the FGF-23 receptor-associated marker (coreceptor) Klotho [15, 16]. Klotho has been associated with anti-atherosclerotic (anti-age)-associated factors [16] and has been proposed as a therapeutic agent in the future [17]. Knockout Klotho mice may achieve similar metabolic disturbances as HD patients [17].

We performed correlation analyses with sclerostin, TNF-alpha, Il-18, and Klotho and the five markers studied earlier [15] to find possible links to calcification and inflammatory vessel disease in HD patients.

Materials and methods

Patients. A total of 84 HD patients with a mean age of 66.3 ± 16.6 (median 71; range 20–90) years were studied (Table 1) together with 34–61 controls depending on the marker analysed (mean age 46 ± 11; median 47; range 22–66) Table 2. Subclassification of the HD patients according to the diagnoses is shown in Table 1. The median parathyroid hormone (PTH) levels of the HD patients at the time of blood sampling were 240 ng/l (normal range 15–65 ng/l).

Ethics. All patients provided informed consent before blood sampling. The study was approved by the ethics committee of Linköping University Hospital, Sweden.

Methods. Sera were stored frozen until use.

Sclerostin, TNF-alpha, Klotho, and IL-18 were measured with ELISA techniques by commercially available

Table 1 Subclassification of haemodialysis patients (*n* = numbers of patients).

Diagnosis	*n*
Nephrosclerosis/uraemia UNS	32
Diabetic nephropathy	17
Glomerulonephritis	12
Polycystic kidney disease	11
Chronic tubulo-interstitial nephritis	4
Malignancy	2
Urinary obstruction (kidney stones)	3
SLE/MPO-vasculitis	2/1
Total	84

UNS, Unknown cause; SLE, Systemic lupus erythematodes; MPO, Microscopic polyangiitis.

kits. For sclerostin, human sclerostin HS EIA kit from TECOmedical group, Quidel Corporation, San Diego, CA, USA, was used. For TNF-alpha Quantikine HS ELISA kit and for Klotho a DuoSet ELISA development system for Human Klotho, both from R&D Systems Europe Ltd., Abingdon, OX, UK, were used. A human IL-18 ELISA Kit from MBL Medical & Biological Laboratories Co., Ltd. (Diegen, Belgium) was used according to the manufacturer's instructions. Parathyroid hormone (PTH) analyses were performed according to the hospital routines and recorded as present at the sampling for the study.

The results of this study were analysed separately but were also compared to those for all the markers from the earlier study [15]. The same patient sera were used for all markers studied [15]. In the controls, fewer sera were used for the additional four markers than in study [15] due to lack of certain sera.

Statistical analyses. All inflammatory markers were log-normally distributed within the two groups and, hence, values were transformed to the natural logarithm. Klotho included zeros and, therefore, ln (x + 0.5) was used. The transformed variables are denoted Ln x. As mean age was significantly different between the two groups [15], group differences were analysed using analyses of covariance (ANCOVA) adjusted for age and gender. Correlation matrices, calculated for the two groups separately, are tabled (Pearson's correlation coefficient and corresponding *P*-values). The data were analysed in Statistica version 12 (Statistica; StatSoft®, Tulsa, OK, USA).

Results

We found significant differences ($P < 0.05$) in the levels of the four new markers; sclerostin, TNF-alpha, IL-18, and Klotho, between HD patients and healthy controls. In the case of Klotho, the controls had higher levels than the patients. Importantly, the differences were still present when the material was adjusted for age and gender (Table 2, Fig 1). In the patient group, Ln sclerostin correlated significantly with gender, Ln TNF-alpha, HGF and suPAR. Ln TNF-alpha correlated significantly with Ln sclerostin, Ln hsCRP, Ln IL-6, Ln FGF-23, Ln suPAR and Ln IL-18. Ln IL-18 correlated significantly with Ln suPAR and Ln TNF-alpha. Ln Klotho correlated significantly with gender and negatively with Ln hsCRP (Tables 2 and 3). In the controls, Ln Sclerostin correlated with age ($P = 0.000$), Ln TNF-alpha correlated with gender ($P = 0.034$), and Ln IL-18 correlated with age ($P = 0.013$) and Ln suPAR ($P = 0.01$).

Discussion

We studied TNF-alpha, sclerostin, IL-18 and Klotho which, in the case of sclerosin, TNF-alpha and IL-18 were found to correlate in low but significant levels both to each

.

Parameter	HD patients	Controls	P-value
Sclerostin_ng/ml			
n	84	61	
Crude			
Mean	2.3	0.6	
Median (range)	1.9 (0.6–8.3)	0.6 (0.3–1.0)	
Ln (mean)	0.67	−0.62	<0.001
Ln (SD)	0.61	0.30	
Age & gender adjusted			
Ln (mean)	0.61	−0.53	<0.001
hsTNF-alpha (pg/ml)			
n	84	48	
Crude			
Mean	4.8	1.6	
Median (range)	4.2 (1.8–24)	1.5 (0.8–3.4)	
Ln (mean)	1.48	0.41	<0.001
Ln (SD)	0.39	0.35	
Age & gender adjusted			
Ln (mean)	1.48	0.42	<0.001
Klotho (pg/ml)			
n	78	39	
Crude			
Mean	878	1501	
Median (range)	4.0 (0.0–23730)	89 (3–17130)	
Ln (mean)	2.23	5.17	<0.001
Ln (SD)	3.15	2.05	
Age & gender adjusted			
Ln (mean)	2.45	4.74	<0.001
Il-18 (pg/ml)			
n	84	34	
Crude			
Mean	383	202	
Median (range)	355 (46–2000)	205 (22–390)	
Ln (mean)	5.77	5.12	<0.001
Ln (SD)	0.61	0.72	
Age & gender adjusted			
Ln (mean)	5.74	5.18	<0.001

Table 2 Serum levels of 4 tested markers in haemodialysis (HD) patients and controls. Crude is measured values and age- & gender-adjusted values are values using analysis of covariance (ANCOVA). Ln is the natural logarithm

other and to other markers tested in a former study [15] in the same patients. Klotho correlated negatively to hsCRP, a marker tested in the former study [15]. TNF-alpha, sclerostin and IL-18 were higher in patients than in controls, while Klotho was higher in the controls than in the patients. Sclerostin correlated to the pro-inflammatory cytokine TNF-alpha but also to suPAR and HGF. SuPAR has been suggested to be a marker of atherosclerotic disease in stroke and may be related to the prognosis in septicaemia [18, 19]. HGF is a factor shown to be of importance in periodontitis and to the acute phase responses in the bowel and lung [20–22]. TNF-alpha levels correlated to the inflammatory markers hsCRP and IL-6, as well as to suPAR and IL-18 but also to the levels of phosphaturic hormone FGF-23, which also may be involved in bone mineralization and resorption and furthermore in vascular calcification [8, 9, 15, 23]. FGF-23 has recently been implicated to be more strongly associated with myocardial than vascular toxicity [24].

Sclerostin has not been studied extensively and may be considered to be a new link to both bone and vessel disease in HD patients [25, 26]. Vascular inflammation may be one of the mechanisms to consider; an important morbidity and mortality factor in HD patients [27, 28]. IL-18, another pro-inflammatory cytokine, correlated to suPAR and TNF-alpha. Angiopoietins and pro-inflammatory cytokines, such as IL-18, may be involved in vascular calcification [29–32]. Liu et al. [32] found elevated IL-18 levels to be associated with all-cause mortality in stable HD patients independently of cardiac dysfunction. The FGF-23 receptor-associated marker Klotho correlated negatively to the inflammatory marker hsCRP but did surprisingly not correlate with FGF-23. Klotho exists, however, both in a membrane-bound and a soluble form [17]. The Klotho levels were as expected lower in patients than in controls. This is consistent with the hypothesis of downregulation of Klotho by FGF-23 and reduced production in chronic renal failure [33–35].
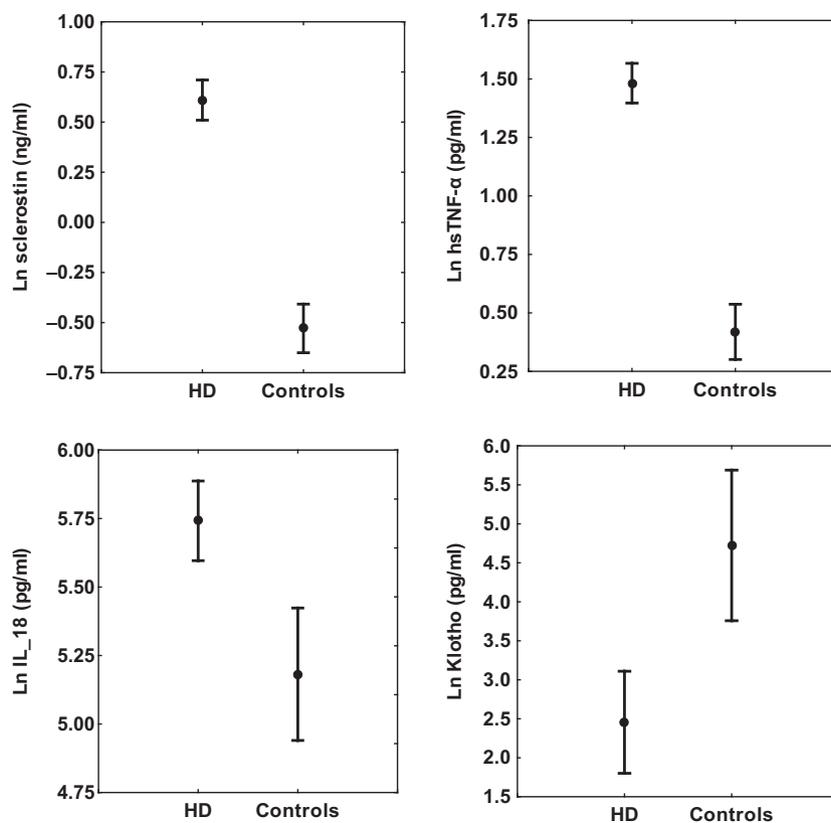
Figure 1 Age- and gender-adjusted values for the four markers; significant differences between patients and controls for all the four markers were $P < 0.001$.

Table 3 Correlations in HD patients and controls of Sclerostin, TNF-alpha, IL-18 and Klotho with all the markers tested previously as well as the age and gender. Ln is the natural logarithm.

	Ln_Sclerostin	Ln_hsTNF-α	Ln_Klotho	Ln_IL-18
HD patients				
Age	0.18 ($P = 0.111$)	−0.02 ($P = 0.862$)	−0.21 ($P = 0.069$)	0.02 ($P = 0.849$)
Gender	**0.29 ($P = 0.007$)**	0.21 ($P = 0.059$)	**0.26 ($P = 0.022$)**	0.15 ($P = 0.181$)
Ln CR P	0.19 ($P = 0.084$)	**0.38 ($P = 0.000$)**	**−0.28 ($P = 0.013$)**	0.10 ($P = 0.385$)
Ln IL-6	0.16 ($P = 0.142$)	**0.22 ($P = 0.047$)**	0.08 ($P = 0.478$)	0.17 ($P = 0.133$)
Ln FGF-23	0.16 ($P = 0.145$)	**0.22 ($P = 0.042$)**	0.11 ($P = 0.351$)	−0.15 ($P = 0.168$)
Ln HGF	**0.35 ($P = 0.001$)**	0.20 ($P = 0.065$)	−0.08 ($P = 0.464$)	0.20 ($P = 0.063$)
Ln suPAR	**0.39 ($P = 0.000$)**	**0.29 ($P = 0.007$)**	0.04 ($P = 0.738$)	**0.38 ($P = 0.000$)**
Ln_Sclerostin	–	**0.40 ($P = 0.000$)**	0.11 ($P = 0.344$)	0.16 ($P = 0.150$)
Ln_hsTNF-α		–	0.05 ($P = 0.692$)	**0.23 ($P = 0.032$)**
LN_Klotho			–	0.09 ($P = 0.452$)
Ln_IL-18				–
Controls				
Age	**0.47 ($P = 0.000$)**	0.14 ($P = 0.350$)	−0.23 ($P = 0.159$)	**0.42 ($P = 0.013$)**
Gender	0.25 ($P = 0.054$)	**0.31 ($P = 0.034$)**	0.12 ($P = 0.459$)	−0.21 ($P = 0.239$)
Ln CRP	0.12 ($P = 0.376$)	0.16 ($P = 0.285$)	−0.08 ($P = 0.648$)	0.17 ($P = 0.324$)
Ln IL-6	0.18 ($P = 0.155$)	0.03 ($P = 0.817$)	−0.20 ($P = 0.215$)	0.08 ($P = 0.657$)
Ln FGF-23	−0.10 ($P = 0.455$)	0.01 ($P = 0.941$)	0.29 ($P = 0.069$)	−0.05 ($P = 0.796$)
Ln HGF	−0.02 ($P = 0.891$)	0.21 ($P = 0.149$)	−0.25 ($P = 0.133$)	0.34 ($P = 0.052$)
Ln suPAR	0.13 ($P = 0.302$)	0.24 ($P = 0.097$)	−0.04 ($P = 0.806$)	**0.44 ($P = 0.010$)**
Ln_Sclerostin	–	0.16 ($P = 0.288$)	−0.03 ($P = 0.843$)	−0.01 ($P = 0.965$)
Ln_hsTNF-α		–	−0.28 ($P = 0.111$)	0.23 ($P = 0.193$)
LN_Klotho			–	−0.17 ($P = 0.410$)
Ln_IL-18				–

Bold text signifies $P<0.05$.

The study suggests that the interplay within reactive factors (cytokines and growth factors) could be possibly related to the progression of vessel disease and the long-term prognosis in HD patients and should be studied further. Sclerostin has been suggested to be linked to bone mineral density in HD patients [2, 3, 35]. It has also been associated with uraemic toxicity and vessel calcification in these patients but not to parathyroid hormone levels [2, 3, 36]. Viaene *et al.* and Drechsler *et al.* found lower mortality in haemodialysis patients with high circulating sclerostin levels [36, 37]. Results on the role for sclerostin in vascular calcification are still conflicting [38, 39]. Future studies may address correlations of the studied markers with cytokines as IL-10 and IL-12 or angiopoietins as vascular endothelial growth factor (VEGF) as well as possible reaction patterns in septicaemia or in vascular calcification. The interactions and biological roles of IL-18 and TNF-alpha have recently been studied by others [40]. Cytokine and growth factor removal with haemodiafiltration or haemodialysis may be of importance in sepsis-related acute kidney injury patients [41]. The importance of vitamin D- and FGF-23 levels in HD patients and the influence which dialysis mode might cause, recently studied by a group from our unit, remains to be elucidated [42]. The FGF-23-Klotho axis and vitamin D substitution is also considered to be a possible diagnostic and therapeutic target [17, 43–46]. The potential vascular effects of Klotho may be of special interest [47–49].

Acknowledgment

Conflict of interest

None to declare

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