

# The association of serum soluble Klotho levels and residual diuresis and overhydration in peritoneal dialysis patients

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D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online)

*Adv Clin Exp Med.* 2019;28(10):1345–1349

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## Funding sources

The study was supported with a Poznan University of Medical Sciences Grant for Young Researchers (No. 502-14-02225363-99663).

## Conflict of interest

None declared

## Acknowledgements

We would like to thank Janusz Witowski for comments that greatly improved the manuscript.

Received on February 25, 2018

Reviewed on May 21, 2018

Accepted on February 18, 2019

Published online on March 11, 2019

## Abstract

**Background.** Klotho, originally identified as an anti-aging factor, is a transmembrane protein expressed in the kidney. It has been reported that Klotho deficiency could be associated with a loss of residual renal function and cardiovascular complications in peritoneal dialysis (PD) patients.

**Objectives.** The main aim of the study was to evaluate whether serum levels of Klotho correlate with residual diuresis and hydration status in PD patients.

**Material and methods.** The cross-sectional study involved 57 PD patients  $\geq 18$  years of age who had been on PD  $\geq 3$  months. Serum Klotho was measured using high-sensitivity enzyme-linked immunosorbent assay (ELISA). Hydration status was assessed with bioimpedance analysis (BIA).

**Results.** Serum levels of soluble Klotho ranged from 100 pg/mL to 700 pg/mL. The patients were divided into 2 subgroups, with Klotho levels below and above the median (260 pg/mL). The data revealed a tendency for lower residual diuresis ( $1.3 \pm 1.0$  L vs  $1.8 \pm 0.8$  L;  $p = 0.055$ ) in patients with lower levels of Klotho in serum. Serum Klotho correlated negatively with overhydration according to BIA ( $r = -0.27$ ;  $p = 0.044$ ) and positively with residual diuresis ( $r = 0.26$ ;  $p = 0.045$ ).

**Conclusions.** Soluble Klotho correlates inversely with hydration status in BIA. Residual urine output, but not dialysis parameters, could be associated with the levels of serum soluble Klotho in PD patients.

**Key words:** peritoneal dialysis, Klotho, overhydration

## Cite as

Sikorska D, Pawlaczyk K, Baum E, et al. The association of serum soluble Klotho levels and residual diuresis and overhydration in peritoneal dialysis patients. *Adv Clin Exp Med.* 2019;28(10):1345–1349. doi:10.17219/acem/104552

## DOI

10.17219/acem/104552

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Klotho was first identified in mice as an anti-aging factor.<sup>1</sup> Full-length alpha-Klotho is a single-pass transmembrane protein that exists in 2 forms, membrane and secreted Klotho, which have different functions.<sup>2</sup> The membrane form acts as a co-receptor for fibroblast growth factor-23 (FGF-23) and plays an important role in calcium-phosphate metabolism.<sup>2,3</sup> Soluble Klotho (called  $\alpha$ -Klotho, with molecular mass 130-kDa) seems to function as a humoral factor with various biological effects, and it works independently of FGF-23 signaling.<sup>4</sup> Among the pleiotropic actions that soluble Klotho is responsible for are tissue protection from oxidative stress, fibrosis and apoptotic stimuli; regulation of blood phosphate and vitamin D3 levels; and the activity of multiple cell surface calcium and potassium ion channels.<sup>2,3,5</sup> There are data suggesting that secreted Klotho exerts phosphaturic effects independently of FGF-23.<sup>3</sup> Soluble Klotho has also been associated with protective effects against vascular calcification.<sup>6</sup> Furthermore, soluble Klotho could be a potential biomarker for predicting adverse renal outcomes in patients with advanced chronic kidney disease (CKD).<sup>7–9</sup> However, the exact diagnostic and therapeutic role of Klotho in humans is not fully known yet.<sup>3</sup>

Klotho is expressed in several organs, including the parathyroid glands, the choroid plexus of the brain and, predominantly, in the distal tubular epithelial cells of the kidney.<sup>10</sup> The kidney is the major source of Klotho in humans, and this organ is involved in Klotho homeostasis, responsible for producing and releasing Klotho into the circulation.<sup>11</sup> Patients with CKD display decreased Klotho gene expression in several tissues, including the kidney. This results in reduced levels of circulating Klotho. Serum soluble Klotho is decreased in all stages of CKD, especially in dialysis patients.<sup>8,11</sup> It is likely that serum soluble Klotho protein concentration is related to residual renal function.<sup>8</sup> However, the relationship between the soluble form of Klotho and residual renal function in chronic peritoneal dialysis (PD) patients remains poorly understood.<sup>7,9</sup>

The aim of this study was to assess whether serum soluble Klotho levels could be associated with residual renal function and hydration status in PD patients. To the best of our knowledge, this is the first study evaluating the relationship between serum Klotho and hydration status as assessed with bioimpedance analysis (BIA) in patients on PD.

## Material and methods

### Patients

This investigator-initiated cross-sectional study involved 57 Caucasian patients undergoing PD in 3 regional dialysis centers. The inclusion criteria were: age  $\geq 18$  years, time on PD  $\geq 3$  months, and informed consent. The exclusion criteria were: any acute inflammatory disease within 12 weeks prior to enrolment, amputated limbs and cardiac pacemakers or implantable cardioverter defibrillators. The study was

approved by the Poznan University of Medical Sciences Bioethics Committee (No. 424/13) and informed consent was obtained from all the participants included in the study.

### Volume status

A peritoneal equilibration test (PET) using a 4-hour dwell of 2.27%-glucose dialysate was used to assess peritoneal membrane transport.<sup>12</sup>

Hydration status was assessed with bioimpedance spectroscopy using the Body Composition Monitor (BCM) (Fresenius Medical Care GmbH, St. Wendel, Germany).<sup>13</sup> The measurements were performed in the supine position under standardized conditions. Values below  $-1.1$  L and above  $+1.1$  L corresponded to hypovolemia and hypervolemia, respectively.<sup>13</sup> Clinical assessments of hydration status were based on blood pressure measurements and on the symptoms of overhydration: the presence of dyspnea, peripheral edema and jugular vein distension.

### Laboratory tests

Samples of serum were collected at the time of the clinical examinations in a fasting state. Serum was aliquoted and stored at  $-80^{\circ}\text{C}$  until assayed in batches. Serum Klotho was measured using the Human Soluble  $\alpha$ -Klotho Assay Kit (Immuno-Biological Laboratories Co. Ltd., Fujioka, Japan) with a sensitivity of 6.15 pg/mL. The immunoassays were performed according to the manufacturer's instructions. All other laboratory tests were performed in the hospital central laboratory using routine methods.

### Statistical methods

Statistical analyses were performed using STATISTICA v. 10.0 software (StatSoft Polska, Kraków, Poland). The normality of the data distribution was checked with the Shapiro–Wilk test. The data is presented as medians and interquartile ranges or percentages, as appropriate. Differences between unpaired data were analyzed with the Mann–Whitney U test. Categorical data was analyzed with the  $\chi^2$  test. Correlations between variables were analyzed with Spearman's rank correlation coefficient. Differences were considered significant at  $p < 0.05$ .

## Results

The study analyzed 57 consecutive PD patients. Serum levels of soluble Klotho ranged from 100 to 700 pg/mL. The patients were divided into 2 subgroups: those with Klotho levels below the median (260 pg/mL) and those with levels above the median. We decided on such subdivision of patients because there are no clear ranges of reference values for Klotho.<sup>4</sup> The patients' characteristics in relation to their serum Klotho levels are shown in Table 1.

Clinical features of overhydration were observed in 19% of the patients (11/57). However, BIA found excessive hydration retention in as many as 44% of the patients (25/57). In addition, there was a significant difference in the distribution of those patients between the Klotho subgroups (Table 1). Surprisingly, there were no statistically significant differences in Klotho levels between patients with overhydration according to BIA and those without (250 (216–327) pg/mL vs 290 (223–399) pg/mL;  $p = 0.172$ ).

The data revealed a tendency toward higher fluid overload in BIA in patients with lower levels of Klotho in serum

(Table 1), although these were not statistically significant differences. Also, patients with lower serum Klotho had significantly lower hematocrit and hemoglobin levels. These patients also tended to have lower residual diuresis. However, there was no relationship between serum Klotho levels and dialysis parameters (Table 1).

As expected, serum Klotho correlated negatively with overhydration according to BIA ( $r = -0.27$ ;  $p = 0.044$ ) (Fig. 1). Moreover, there was a positive correlation between Klotho and residual diuresis ( $r = 0.26$ ;  $p = 0.045$ ) (Fig. 2).

**Table 1.** Patients’ characteristics in relation to serum Klotho levels. Data are presented as medians (interquartile ranges) or as percentages

Variables	Lower levels of Klotho (<260 pg/mL) (n = 28)	Higher levels of Klotho (≥260 pg/mL) (n = 29)	Mann–Whitney or $\chi^2$ test
<b>Demographic and PD-related parameters</b>			
Men [%]	14 (50)	14 (48)	0.896
Age [years]	60 (42–70)	55 (39–67)	0.375
BMI [kg/m <sup>2</sup> ]	24.4 (21.0–30.3)	26.0 (23.9–29.1)	0.664
Diabetic nephropathy, n [%]	8 (29)	7 (24)	0.704
DM [%]	11 (39)	9 (31)	0.514
Time on PD [months]	25 (15–38)	33 (16–68)	0.123
APD mode, n [%]	10 (36)	6 (21)	0.207
Ultrafiltration [mL/day]	1,200 (500–2,200)	1,000 (900–1,500)	0.766
Residual diuresis [mL/day]	1,300 (500–2,200)	1,650 (1,100–2,400)	0.054
Residual diuresis <500 mL/day [%]	7 (25)	1 (3)	0.019
Solute removal [Kt/V]	2.3 (2.1–3.5)	2.8 (2.3–3.3)	0.421
Creatinine clearance [L/week]	92.8 (75.4–120.2)	99.4 (68.8–136.1)	0.799
4-h D/P creatinine in PET	0.68 (0.58–0.72)	0.63 (0.55–0.71)	0.402
Transport status, n (% H/HA)	16 (57)	12 (41)	0.234
<b>Blood tests and biochemical parameters</b>			
Hematocrit [%]	33.1 (31.5–37.4)	37.1 (33.4–38.5)	0.018
Hemoglobin [g/dL]	11.1 (10.5–12.4)	12.5 (11.3–13.4)	0.016
CRP [mg/L]	5.6 (2.6–8.4)	2.7 (1.3–8.2)	0.123
Albumin [g/dL]	3.8 (3.5–4.1)	4.0 (3.7–4.2)	0.107
Total cholesterol [mg/dL]	184 (167–201)	198 (169–216)	0.263
Calcium [mmol/L]	9.1 (8.5–9.5)	9.0 (8.7–9.3)	0.936
Phosphorus [mmol/L]	5.3 (3.9–6.2)	5.1 (4.3–6.1)	0.707
PTH [pg/mL]	278 (178–401)	340 (299–490)	0.080
FGF-23 [pg/mL]	8.9 (3.2–41.6)	18.3 (5.6–44.4)	0.457
<b>Hydration status</b>			
OH in BIA [L]	2.0 (0.6–3.0)	0.8 (0.2–1.6)	0.066
OH in BIA [%]	2.3 (0.9–3.9)	1.0 (0.3–2.5)	0.056
Overhydration in BIA > 1.1 L [%]	16 (57)	9 (31)	0.047
Pts with edema, n [%]	7 (25)	4 (14)	0.284
SBP [mm Hg]	130 (120–150)	130 (120–145)	0.705
DBP [mm Hg]	80 (70–95)	80 (70–90)	0.497
Number of antihypertensives	3 (2–4)	4 (2–4)	0.215

BMI – body mass index; DM – diabetes mellitus; PD – peritoneal dialysis; APD – automated peritoneal dialysis; H – high; HA – high-average; CRP – C-reactive protein; PTH – parathyroid hormone; FGF-23 – fibroblast growth factor 23; OH – overhydration; BIA – bioimpedance analysis; SBP – systolic blood pressure; DBP – diastolic blood pressure.

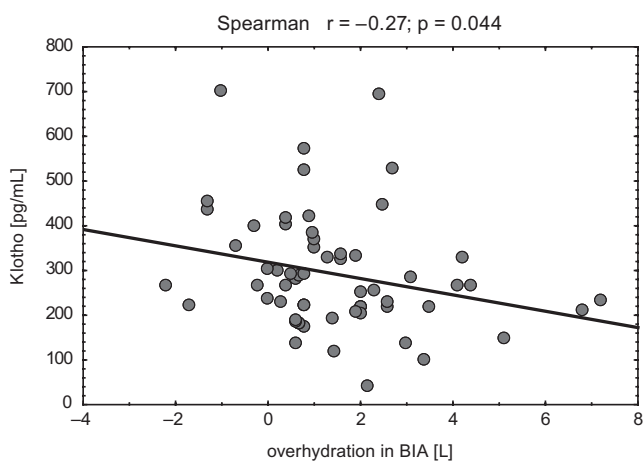


Fig. 1. Correlation between hydration status in bioimpedance analysis (BIA) and serum Klotho

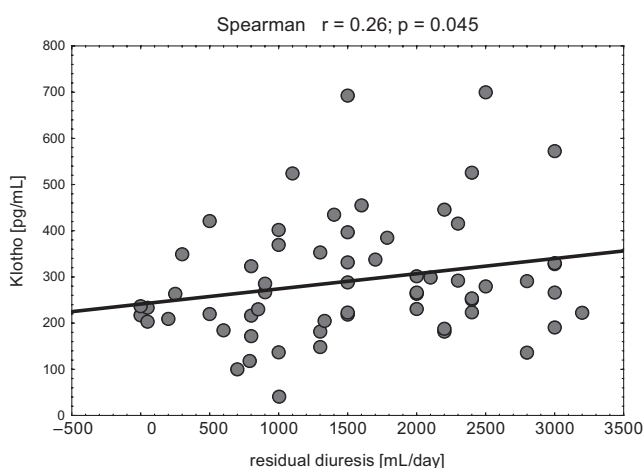


Fig. 2. Correlation between residual diuresis and serum Klotho

## Discussion

The most important observation in our study was that serum soluble Klotho levels correlate inversely with hydration status in BIA. The results of the study suggest that residual urine output, but not dialysis parameters, could be associated with the levels of serum Klotho in PD patients.

Earlier studies indicated that serum soluble Klotho levels are positively associated with renal function and are significantly decreased in the later stages of CKD.<sup>14,15</sup> At the same time, Klotho concentrations are probably independent of PD parameters themselves.<sup>16</sup> However, the relationship between serum Klotho and residual renal function is not so obvious in PD patients.<sup>7,9</sup> A previous study revealed that the total amount of urinary excreted Klotho, but not the serum level of soluble Klotho, may be a potential biomarker for assessing the residual renal function among PD patients.<sup>9</sup> In 2012, Golembiewska et al. demonstrated that serum soluble Klotho concentrations

were negatively correlated with a 24-hour diuresis, but not with residual renal function.<sup>7</sup>

There has been a strong focus on residual renal function as a significant survival predictor for dialysis patients,<sup>17,18</sup> but the precise mechanism by which residual diuresis is linked to morbidity and mortality among dialysis patients has yet to be determined.<sup>19</sup> The presence of residual renal function is associated with better preservation of the renal endocrine and metabolic functions, and facilitates the maintenance of good hydration status.<sup>20,21</sup> It is likely that residual urinary volume is the main predictor of overhydration in patients on PD.<sup>22</sup> Moreover, our study demonstrated a significant correlation between residual urine output and hydration status ( $r = -0.36$ ;  $p = 0.004$ ).

It is possible that the relationship between residual diuresis and soluble Klotho in our study results from hydration status. However, to rule out the effect of dilution of serum Klotho, albumin concentrations were also assessed, and there was no relationship between Klotho levels and albumin concentrations. At the same time, we did not detect any correlation between overhydration in BIA and blood pressure, which should be influenced by an increase in plasma volume. This may suggest that overhydration is related to interstitial fluid retention rather than hypervolemia.<sup>23</sup> It is possible that overhydration per se affects soluble Klotho levels.

The introduction of BIA has made it possible to assess hydration status in PD patients more accurately.<sup>13</sup> Recent studies have revealed that BIA-evaluated overhydration is common in dialysis patients, and is associated with loss of residual renal function and inflammation.<sup>24,25</sup> Increasing evidence suggests that overhydration predicts all-cause mortality.<sup>26</sup> However, the relationship between overhydration and Klotho is not yet known. To the best of our knowledge, this is the first study evaluating the relationship between serum Klotho and hydration status in BIA in patients on PD.

Although this study provides new information on the relationship between residual diuresis, hydration status and soluble Klotho among PD subjects, the results should be interpreted within the context of the study limitations. The patient population was relatively small, which means that the study may be statistically underpowered. An additional limitation is that we cannot provide data on urinary and dialysate Klotho excretion. Further research is therefore needed.

## Conclusions

Residual urine output, but not dialysis parameters, could be associated with the levels of serum soluble Klotho in PD patients. Soluble Klotho correlates inversely with hydration status in BIA.

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