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Exploratory Pilot Study of Exercise-Associated Klotho Modulation in Hemodialysis Patients

Klotho, Exercise, and Anti-Aging in CKD

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Abstract

Background: Chronic kidney disease (CKD) is characterized by accelerated aging and decline in physical function. Klotho, an anti-aging protein predominantly expressed in the kidney, plays a crucial role in mineral metabolism and longevity. Exercise has been proposed as a non-pharmacological strategy to enhance Klotho expression; however, clinical evidence in hemodialysis patients remains limited. Objective: This study aimed to explore the association between exercise and plasma Klotho levels using a combined case study and cross-sectional design. Methods: This study included: (1) A prospective case study evaluating the effects of high-intensity interval training (HIIT) in a hemodialysis patient. (2) A cross-sectional analysis comparing plasma Klotho levels between hemodialysis patients (n=24) and healthy controls (n=18) and assessing their association with habitual physical activity. Plasma Klotho levels were measured using ELISA. Statistical analyses included the Mann–Whitney U test and Spearman's correlation coefficient. Results: In the case study, improvements in muscle strength and exercise tolerance were observed following HIIT, allowing the patient to resume daily occupational activities. In the cross-sectional analysis, plasma Klotho levels were significantly lower in hemodialysis patients than in healthy controls (p=0.0001). A moderate positive correlation was observed between exercise habits and plasma Klotho levels in hemodialysis patients (r=0.52, p=0.02), whereas no significant association was found in healthy individuals. Conclusion: These findings suggest that exercise therapy may exert potential anti-aging implications in hemodialysis patients through modulation of Klotho expression. This study provides translational evidence linking clinical rehabilitation and molecular aging pathways.

Keywords: Klotho; hemodialysis; exercise therapy; high-intensity interval training; chronic kidney disease; anti-aging; renal rehabilitation

1. Introduction

Chronic kidney disease (CKD) is increasingly recognized as a state of accelerated aging, characterized by sarcopenia, vascular dysfunction, and increased mortality. In Japan, CKD affects approximately one in seven to eight adults[1], and the number of elderly patients undergoing dialysis continues to rise according to the annual statistical survey by the Japanese Society for Dialysis Therapy [2].

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Renal rehabilitation, including exercise therapy, has been shown to improve physical function and quality of life [3,4]. Beyond functional outcomes, recent attention has focused on the molecular mechanisms underlying these benefits [5].

Plasma Klotho (α-Klotho) is a well-established anti-aging protein predominantly expressed in the distal renal tubules [6]. It regulates phosphate metabolism, oxidative stress, and endothelial function[7]. Reduced Klotho expression is associated with CKD progression, cardiovascular disease, and mortality[8].

Emerging evidence suggests that exercise may increase circulating Klotho levels, indicating a potential anti-aging mechanism [9]. However, clinical data in hemodialysis patients remain scarce [10].

Therefore, this study aimed to explore the association between exercise and plasma Klotho levels and contribute to potential anti-aging implications in CKD patients using a combined case study and cross-sectional approach.

2. Methods

2.1. Study Design:

This study consisted of two components:

(1) a prospective case study evaluating the effects of high-intensity interval training (HIIT), and (2) a cross-sectional study investigating the association between exercise habits and plasma Klotho levels.

2.2. Participants

Case Study: The case study involved a male patient (table1) in his 60s undergoing maintenance hemodialysis for more than 10 years. For the case study, the patient was selected as a representative case from a consecutive cohort of patients who initiated the HIIT protocol, based on his clinical requirement for functional recovery. The primary renal disease was IgA nephropathy. The patient had stable hemodynamic status and was deemed eligible for rehabilitation by the attending physician. The patient was receiving standard medications, including antihypertensive agents. No changes in regular medications were made before or after the intervention period.

2.3. Cross-Sectional Study

The cross-sectional study included 24 patients undergoing maintenance hemodialysis and 18 healthy controls. Participants were recruited using a consecutive sampling method. All hemodialysis patients who visited the clinic during the study period and met the inclusion criteria were invited to participate to minimize selection bias.

2.4. Exercise Intervention (Case Study)

The HIIT program consisted of resistance and aerobic training, following established protocols for renal rehabilitation [11]. Resistance training was performed using power rehabilitation machines. The HIIT program consisted of resistance and aerobic training. Resistance training was performed using power rehabilitation machines targeting knee flexion/extension and hip abduction/adduction. The protocol included:

Low-intensity exercise: 10 repetitions × 2 sets at 11 on the Borg Rating of Perceived Exertion (RPE) scale

High-intensity exercise: 10 repetitions × 1 set at 14–15 RPE [12]

Aerobic exercise was conducted using a cycle ergometer [11]:

Low-intensity intervals: 2 minutes × 4 sets at 11–13 RPE

Moderate-to-high intensity intervals: 1 minute × 2 sets at 13–14 RPE

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In addition, a 5-minute whole-body stretching routine was performed as part of both the warm-up and cool-down phases.

2.5. Measurement of Klotho

Klotho levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Immuno-Biological Laboratories Co., Ltd., Japan), according to the manufacturer's instructions. This assay has been previously validated for its precision and reliability in clinical populations [13,14]. Plasma samples were processed and analyzed following the standardized protocol [15], and values were expressed in pg/mL.

2.6. Assessment of Exercise Habits

Exercise habits were assessed through structured interviews. Participants were asked about the type, duration, and frequency of their usual physical activities. Exercise intensity was quantified using the Compendium of Physical Activities [16], and MET values were calculated accordingly to estimate physical activity levels [17,18].

2.7. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (version 27.0; IBM Corp., Armonk, NY, USA) [19]. Group comparisons were performed using the Mann–Whitney U test. Associations between exercise habits and plasma Klotho levels were analyzed using Spearman's rank correlation coefficient. The area under the curve (AUC) was calculated to assess the predictive power of the model [20]. A p-value < 0.05 was considered statistically significant.

2.8. Sample Size Calculation

This study was designed as an exploratory pilot study. The sample size was determined based on feasibility and prior similar studies investigating Klotho levels and exercise interventions in CKD populations[21,22]. In the cross-sectional analysis, a sample size of approximately 20 participants per group is considered sufficient to detect moderate to large correlations ($r \geq 0.5$) with a statistical power of 0.8 and a significance level of 0.05 [23]. Therefore, the present sample size (hemodialysis: n=24, controls: n=18) was considered appropriate for detecting clinically meaningful associations. Although the sample size was determined based on previous studies and a power of 0.8, it remains relatively small for a cross-sectional analysis. Therefore, this study is positioned as an exploratory pilot study, and while the results provide meaningful preliminary insights, the generalizability of the findings should be interpreted with caution.

2.9. Rationale for Study Design

A combined case study and cross-sectional design was adopted to provide both mechanistic and clinical insights[24]. The case study allowed detailed evaluation of the effects of a structured HIIT intervention on functional outcomes in a real-world clinical setting [25]. The cross-sectional component enabled the investigation of associations between habitual physical activity and plasma Klotho levels in a broader population. This combined design was chosen to bridge individual-level intervention effects and population-level associations, thereby providing a translational perspective in renal rehabilitation [26].

2.10. Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki[27]. The study protocol was reviewed and approved by the Research Ethics Committee of Tsukuba University of Technology (Approval No. 2023-29, approved on November 7, 2023). Written informed consent was obtained from all participants prior to participation. All procedures were performed in accordance with relevant guidelines and regulations.

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3. Results

In the case study, the HIIT intervention led to a measured functional recovery. The HIIT program was completed without any adverse events, such as intradialytic hypotension or excessive fatigue. The patient's Barthel Index improved from 90 to 100, and the SPPB score increased from 10 to 12. Peak exercise intensity rose from 2.0 to 5.5 METs (Table 1), and the patient resumed agricultural work. In the cross-sectional analysis, plasma Klotho levels were significantly lower in hemodialysis patients than in healthy controls ($p = 0.0001$). The analysis confirmed low intra-assay variability, supporting the reliability of the ELISA measurements. A moderate positive correlation was observed between exercise habits and plasma Klotho levels in hemodialysis patients ($r = 0.52$, $p = 0.02$), whereas no significant association was found in healthy individuals. Receiver operating characteristic (ROC) analysis in hemodialysis patients using a cutoff value of 450 pg/mL for plasma Klotho demonstrated high sensitivity (1.00) but low specificity (0.125) for identifying individuals with habitual exercise (≥ 3 METs). The Youden index was 0.125, indicating limited discriminative ability of this cutoff value. These findings show that although plasma Klotho levels are associated with exercise habits in hemodialysis patients, a single cutoff value may not be sufficient for accurate clinical classification.

4. Discussion

This study provides novel insights into the potential anti-aging implications of exercise therapy in hemodialysis patients by focusing on Klotho modulation. The case study demonstrated that HIIT can substantially improve physical function, even in patients with long-term dialysis[28], While the patient's physical function had previously declined during periods of self-monitored home exercise, the structured HIIT intervention provided sufficient stimulus to overcome "frailty" and enable a return to demanding agricultural work[29]. Specifically, the patient's Barthel Index (BI) score improved from 90 to 100, and the SPPB score increased from 10/12 to a full mark of 12/12[26]. Importantly, the cross-sectional findings revealed a significant association between exercise habits and Klotho levels specifically in hemodialysis patients [30]. Although the age differences and diabetes prevalence between groups are recognized as study limitations, our ROC analysis suggested that while Klotho levels are sensitive to physical activity, a single cutoff value might have limited practical utility for clinical screening due to low specificity. Nevertheless, the significant correlation ($r = 0.52$) and the clinical recovery observed in the case study suggest that Klotho remains a meaningful molecular indicator that reflects the benefits of habitual exercise [31,32]. These clinical observations suggest a bidirectional link: higher habitual activity may help maintain Klotho levels, which in turn supports the anti-aging mechanisms necessary for maintaining high-intensity exercise tolerance[33]. From a translational perspective, this study bridges the gap between macro-level functional recovery (e.g., BI and METs) and micro-level molecular stability (Klotho expression)[34]. These findings highlight the potential of exercise therapy, particularly HIIT, as a potent anti-aging intervention in the management of CKD.

This study has several limitations. First, there was a significant age difference between the hemodialysis group and the healthy control group, which may have influenced the baseline Klotho levels. Despite this mismatch, this exploratory pilot study provides important preliminary evidence regarding the correlation between physical activity and Klotho modulation specifically within the dialysis population. Second, the ROC analysis revealed that while Klotho levels are sensitive to exercise habits, the specificity was notably low (0.125). This suggests that plasma Klotho levels alone may have limited practical utility as a standalone clinical screening tool for physical activity. Nevertheless, the moderate correlation ($r = 0.52$) and the dramatic functional recovery observed in our case study suggest that Klotho remains a meaningful molecular indicator that reflects the biological benefits of habitual exercise.

Conclusion

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Exercise therapy, particularly high-intensity interval training, may improve physical function and may be associated with Klotho modulation in hemodialysis patients. These findings support the integration of molecular biomarkers into renal rehabilitation strategies.

Author Contributions: M.M. (Misa Miura) contributed to exercise intervention, data collection, data analysis, and manuscript writing and is the corresponding author. O.I. (Osamu Ito) and M.K. (Masahiro Kohzuki) contributed to study supervision and scientific validation. S.O. (Shigeru Owada) contributed to clinical coordination and patient management. N.E. (Nobuyuki Endo) performed biochemical analysis. T.M. (Teruhiko Maenami) contributed to medical diagnosis and patient evaluation. All authors have read and approved the final manuscript.

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Data Availability Statement: All data generated or analyzed during this study are included in this published article and its Supplementary Materials.:

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Conflicts of Interest: The authors declare no conflict of interest.

Table 1. Clinical Progress and Outcomes of the Case Study Patient.

Variable	Pre-intervention	Post-intervention (HIIT)
Barthel Index (BI)	90/100	100/100
SPPB score	10/12	12/12 (Full score)
Max Exercise Intensity	2.0 METs	5.5 METs
MMT (Lower extremity)	4	4~5
Lower back pain (NRS)	5~6/10	0/10 (Disappeared)
Blood Pressure	Stable	Stable (No change)
α-Klotho (pg/mL)	342.2	517.9

Table 2. Baseline Characteristics of Participants in the Cross-sectional Study.

Variable	Hemodialysis (n=24)	Healthy Controls (n=18)	p-value
Age (years)	75.5 ± 6.8	50.7 ± 11.4	0.001
Male (%)	25%	16.7%	0.64
Prevalence of diabetes	40%	0	N/A
Primary disease	・Diabetic Nephropathy ・IgA Nephropathy ・Nephrosclerosis ・Polycystic kidney disease ・Idiopathic	-	N/A
α-Klotho (pg/mL)	446.6±174.6	858.8 ±361.2	0.0001

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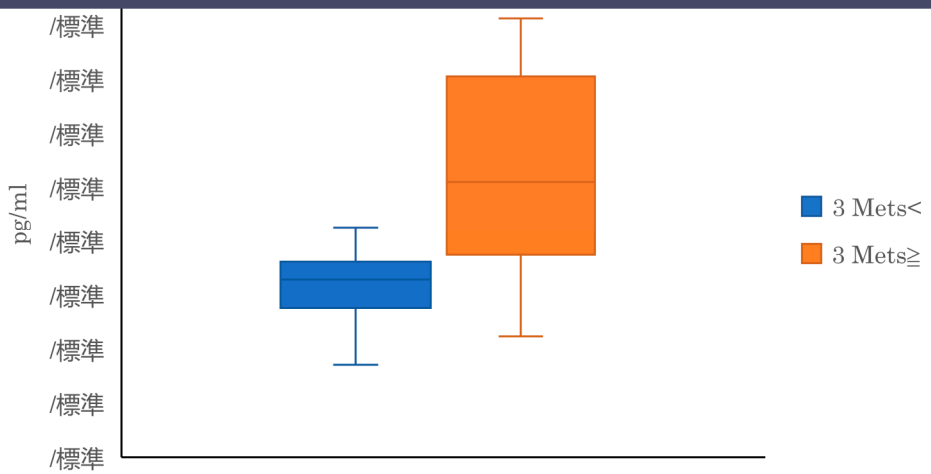

Figure 1. Plasma Klotho protein levels.

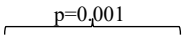

Figure 2. Plasma Klotho levels in dialysis patients with and without habitual physical activity (≥3 METs).

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