HUE UNIVERSITY
UNIVERSITY OF MEDICINE AND PHARMACY

NGUYEN MINH QUAN

# SERUM KLOTHO LEVELS AND THEIR ASSOCIATION WITH MINERAL AND BONE DISORDERS IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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**Pro. VO TAM**

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## 1. INTRODUCTION

Chronic kidney disease (CKD) is a major global health issue with rapidly increasing morbidity and mortality. In 2017, an estimated 843.6 million individuals were affected by CKD; among adults aged ≥ 20 years, the prevalence was 10.4% in men and 11.8% in women. CKD is particularly common in the elderly due to the age-related decline in glomerular filtration rate (GFR) and is strongly associated with diabetes, hypertension, and race-related factors. According to the Global Burden of Disease (GBD) report, CKD-related mortality increased by 41.5% from 1990 to 2017; CKD ranked 12th among causes of reduced life expectancy in 2017 and is projected to rise to 5th place by 2040.

CKD results in a wide range of complications, among which chronic kidney disease–mineral and bone disorder (CKD-MBD) is especially important, as it contributes significantly to fracture risk and cardiovascular mortality. Early detection of CKD and CKD-MBD is therefore crucial for disease control and reducing the healthcare burden.

Klotho, a protein predominantly secreted by renal tubular cells, has recently emerged as a potential biomarker of interest, given its close association with the endocrine function of the kidney. Klotho acts as an obligate co-receptor for fibroblast growth factor 23 (FGF23), thereby participating in the regulation of phosphate and vitamin D metabolism. Numerous studies have reported early reductions in Klotho levels in CKD and associations with CKD-MBD; however, some investigations have failed to confirm these findings. Variability in study designs and existing research gaps have led to ongoing debate regarding the clinical significance of this biomarker.

In Vietnam, research on CKD-MBD has largely focused on calcium, phosphate, parathyroid hormone (PTH), and vitamin D, while data on Klotho and bone mineral density remain limited. For this reason, we conducted the study entitled **"Serum Klotho levels and their association with mineral and bone disorders in patients with chronic kidney disease"**, with the following objectives:

*1. To determine serum Klotho levels in patients with chronic kidney disease stages 1–5.*

*2. To analyze the associations between serum Klotho levels and:*
*a) estimated glomerular filtration rate (eGFR) in predialysis CKD patients;*
*b) mineral–bone parameters (serum calcium, phosphate, PTH, and 25-hydroxyvitamin D) and bone mineral density.*

## 2. CONTRIBUTIONS OF THE DISSERTATION

This is the first study in Vietnam to investigate serum Klotho levels and their association with mineral–bone disorders and bone mineral density in patients with chronic kidney disease.

**Scientific significance:**

The study clarifies how serum Klotho levels change across CKD stages and relate to mineral–bone abnormalities and bone density, improving understanding of CKD–MBD and highlighting the biomarker's potential prognostic value.

**Practical significance:**

The study supports early detection of CKD progression through declining Klotho levels, helps identify the risk of CKD–MBD, and suggests combining Klotho with standard mineral–bone markers for clinical management. The findings also guide early monitoring and interventions to reduce skeletal and cardiovascular complications.

## 3. STRUCTURE OF THE DISSERTATION

The dissertation comprises 136 pages: Introduction (3 pages), Literature Review (38 pages), Subjects and Methods (29 pages), Results (29 pages), Discussion (34 pages), Conclusion (2 pages), and Recommendations (1 page). It includes 42 tables, 10 figures, 1 diagram, 15 charts, and 157 references (10 Vietnamese, 147 English)

# Chapter 1
# LITERATURE REVIEW

## 1.1. CHRONIC KIDNEY DISEASE AND CKD-RELATED MINERAL AND BONE DISORDERS

### 1.1.1. Diagnosis of CKD and Its Complications

*1.1.1.1. Diagnosis of CKD*

According to KDIGO 2012, chronic kidney disease (CKD) is defined by the presence of kidney structural damage and/or decreased kidney function lasting for at least 3 months, with implications for health.

Decreased kidney function is diagnosed when the estimated glomerular filtration rate (eGFR) is ≤ 60 mL/min/1.73 m². Structural kidney damage can be detected directly by kidney biopsy or indirectly through markers such as the urinary albumin-to-creatinine ratio or proteinuria, abnormal urinary sediment, imaging abnormalities, disturbances in fluid and electrolyte balance, tubular dysfunction, or a history of kidney transplantation. CKD is diagnosed when at least one of these two criteria is present, even when eGFR is > 60 mL/min/1.73 m².

*1.1.1.2. Complications of Chronic Kidney Disease*

CKD is associated with multiple severe complications, particularly as eGFR declines. Hypertension is common, accelerating disease progression and increasing cardiovascular risk. Cardiovascular disease—including atherosclerosis, left ventricular hypertrophy, and vascular calcification—remains the leading cause of death. Other major complications include anemia due to reduced erythropoietin production, fluid overload with edema and hypertension, metabolic acidosis, and electrolyte disturbances. Uremic syndrome, manifested by pruritus, fatigue, and sleep disturbances, significantly impairs quality of life. Endocrine dysfunction and chronic inflammation further contribute to systemic complications. Notably, CKD–MBD increases the risk of osteoporosis and fractures and is also associated with higher cardiovascular mortality. Therefore, early detection and appropriate management of CKD–MBD are essential components of comprehensive CKD care.

### 1.1.2. Chronic kidney disease–mineral and bone disorder

*1.1.2.1. Definition of chronic kidney disease–mineral and bone disorder*

In 2005, KDIGO defined CKD–MBD as a systemic disorder of mineral and bone metabolism due to CKD, characterized by one or more of the following: (1) abnormalities in calcium, phosphate, parathyroid hormone, or vitamin D metabolism; (2) abnormalities in bone turnover, mineralization, volume, linear growth, or strength; and (3) vascular or soft-tissue calcification.

*1.1.2.2. Mineral Metabolism Disorders in chronic kidney disease*

CKD–MBD develops early in the course of CKD and becomes more clinically apparent as eGFR declines, particularly below 45–50

mL/min/1.73 m². It is characterized by secondary hyperparathyroidism, hyperphosphatemia, hypocalcemia, and disturbances in vitamin D metabolism. In the early stages, compensatory increases in phosphate excretion by the remaining nephrons help maintain phosphate homeostasis, largely mediated by elevated FGF23 and PTH. However, as eGFR falls below 30–40 mL/min/1.73 m², these adaptive mechanisms become insufficient, resulting in hyperphosphatemia, reduced calcitriol levels, and progressive secondary hyperparathyroidism.

FGF23 (fibroblast growth factor 23), produced mainly by osteocytes and osteoblasts, acts in concert with its co-receptor Klotho in the kidney to promote phosphaturia and suppress renal $1,25(OH)_2D_3$ synthesis. Klotho deficiency attenuates FGF23 signaling, thereby impairing phosphate regulation. Reduced calcitriol further decreases calcium-sensing receptor (CaSR) and vitamin D receptor (VDR) activation, leading to increased PTH secretion. Although PTH initially enhances renal calcium reabsorption and phosphate excretion, sustained elevation promotes bone resorption and contributes to hypercalcemia, hyperphosphatemia, and vascular calcification.

Collectively, the interplay among FGF23, PTH, calcitriol, and Klotho constitutes a progressive pathophysiological axis underlying CKD–MBD, underscoring the importance of early recognition and comprehensive management.

## 1.2. THE ROLE OF KLOTHO IN CHRONIC KIDNEY DISEASE AND ITS RELATIONSHIP WITH MINERAL–BONE DISORDERS

### 1.2.1. Functions of Klotho in chronic kidney disease

*1.2.1.1. Overview of Klotho*

Klotho is predominantly expressed in the kidney as a transmembrane protein that serves as a co-receptor facilitating FGF23 binding to fibroblast growth factor receptors (FGFRs). Its extracellular domain, consisting of two homologous subunits (Kl1 and Kl2), can be cleaved by the proteases ADAM10 and ADAM17, generating soluble Klotho or Kl1/Kl2 fragments that are released into the circulation. Soluble Klotho is detectable in blood, cerebrospinal fluid, and urine.

*1.2.1.2. Methods for measuring serum Klotho levels*

There are three main methods for measuring serum Klotho levels: enzyme-linked immunosorbent assay (ELISA), time-resolved fluorescence immunoassay (TRF), and immunoprecipitation–immunoblot (IP–IB). Among these, ELISA is the most commonly used in clinical practice due to its simplicity, relatively short processing time, and cost-effectiveness.

*1.2.1.4. Functions and pathophysiology of Klotho in chronic kidney disease*

As a co-receptor for fibroblast growth factor 23 (FGF23), the FGFR–Klotho complex mediates key effects on mineral metabolism. In the kidney, it reduces the expression of the NaPi-2a cotransporter, thereby enhancing urinary phosphate excretion. In addition, it suppresses 1α-hydroxylase activity, leading to decreased synthesis of $1,25(OH)_2D_3$. In the distal convoluted tubule, Klotho also regulates the TRPV5 (transient receptor potential vanilloid 5) channel to promote calcium reabsorption and enhances sodium reabsorption via the NCC ($Na^+$–$Cl^-$ cotransporter).

Soluble Klotho, released into the circulation, may reflect the activity of membrane-bound Klotho. This soluble form can also regulate TRPV5 in an FGF23-independent manner by stabilizing the channel on the cell membrane, thereby increasing distal tubular calcium reabsorption. Moreover, soluble Klotho has been reported to exert protective effects, including antioxidant and anti-fibrotic actions, endothelial protection, ion channel regulation, and inhibition of insulin-like growth factor-1 (IGF-1) signaling. These mechanisms may contribute to anti-aging effects and help preserve renal function.

## 1.3. CURRENT RESEARCH STATUS

### 1.3.1. International Studies

Studies in predialysis CKD patients have consistently demonstrated a decline in circulating Klotho levels with worsening kidney function. Pavik et al. (2013) reported that serum Klotho decreased from 1078.6 ± 1810.2 pg/mL in healthy controls to 460.2 ± 222.8 pg/mL in patients with CKD stage 5. Similarly, Rotondi et al. (2015) found significantly lower Klotho levels in CKD patients (519 ± 183 pg/mL) compared with

controls, with reductions observed as early as CKD stage 2. Several studies have reported a positive correlation between serum Klotho and eGFR, as well as associations with mineral–bone markers such as calcium, phosphate, PTH, and FGF23 (Rotondi et al., 2015; Khodeir et al., 2019). However, findings remain inconsistent. Ozeki et al. (2014) did not observe significant correlations between Klotho and calcium or phosphate, while Edmonston et al. (2024) reported that Klotho was not associated with mortality or CKD progression, whereas FGF23 remained an independent predictor. To date, evidence regarding the relationship between Klotho and bone mineral density remains limited and inconclusive.

In maintenance hemodialysis populations, multiple studies have shown lower Klotho levels compared with healthy controls. Ling Yu et al. (2018) reported a mean Klotho level of 379.93 ± 143.66 pg/mL, with no correlation with calcium or PTH and only a weak inverse correlation with phosphate. Wei et al. (2019) observed markedly lower Klotho levels (119.10 ± 47.29 pg/mL) and found no significant associations with calcium, phosphate, or PTH. Similarly, Pasaoglu et al. (2021) reported no significant correlations between Klotho and mineral metabolism markers or vitamin D levels.

### 1.3.2. Domestic Studies

In Vietnam, available evidence remains limited. Nguyen Huu Vu Quang and Vo Tam (2020) reported elevated FGF23 levels in patients with advanced CKD and those receiving hemodialysis. Nguyen Thanh Minh (2021) described a high prevalence of CKD–MBD among maintenance hemodialysis patients, with only 4.91% meeting at least three KDIGO target criteria. More recently, Nguyen Van Chi (2024) reported lower plasma Klotho levels in maintenance hemodialysis patients compared with controls; however, the difference was not statistically significant ($p > 0.05$).

# Chapter 2
# STUDY PARTICIPANTS AND METHODS

## 2.1. STUDY PARTICIPANTS

### 2.1.1. Study period and setting

The study was conducted from October 2022 to June 2024 at Thong

Nhat Hospital. Participants were recruited from the Nephrology Outpatient Clinic (Department of Outpatient Services) and the Department of Nephrology–Hemodialysis. Laboratory analyses were performed at the Departments of Hematology and Biochemistry, and bone mineral density measurements were conducted at the Department of Diagnostic Imaging.

**2.1.2. Patient group**

Inclusion criteria: Patients with CKD stages 1–5 not on dialysis and patients receiving maintenance hemodialysis, who were treated at the Nephrology Outpatient Clinic (Department of Outpatient Services) and the Department of Nephrology–Hemodialysis, Thong Nhat Hospital; aged ≥18 years; and providing written informed consent.

Exclusion criteria: Patients were excluded if they had severe comorbidities or conditions potentially affecting Klotho levels, including acute kidney injury, systemic lupus erythematosus, malignancy, severe infection, endocrine disorders, hepatitis, or cirrhosis. Patients with stage 5 chronic kidney disease not yet on dialysis and eGFR < 7 mL/min/1.73 m² were also excluded. In addition, pregnant or breastfeeding women, patients receiving medications that may influence Klotho levels or bone mineral density (long-term corticosteroids, bisphosphonates, denosumab, or high-dose vitamin D), those who had undergone bone surgery or joint replacement within the previous 6 months, and those with contraindications to bone mineral density measurement were excluded.

**2.1.3. Control group**

Controls were individuals undergoing routine health check-ups at the Nephrology Outpatient Clinic (Department of Outpatient Services), Thong Nhat Hospital, with normal clinical examination and basic laboratory test results (complete blood count; blood glucose; renal function; liver enzymes; electrolytes including sodium, potassium, chloride, and calcium; urinalysis using 10 parameters; electrocardiography; chest X-ray; and abdominal ultrasound), meeting no exclusion criteria and providing informed consent.

**2.2. STUDY CONTENTS AND METHODS**

**2.2.1. Study design**

This was a cross-sectional descriptive study with analytical components.

**2.2.2. Sample size calculation**

The minimum required sample size for each group was calculated based on the comparison of mean serum Klotho levels between the CKD group and the control group.

$$n = \frac{2 \text{ x } C}{(ES)^2}$$

According to Rotondi, the mean serum Klotho level in CKD patients was 519 ± 183 pg/mL, which was lower than that in controls (845 ± 330 pg/mL). With α = 0.05 and power = 95% (β = 0.05), the constant C = 13.0. The calculated sample size was approximately 27 participants per group. This study included 300 participants: 60 healthy controls, 150 predialysis CKD patients (stages 1–5), and 90 maintenance hemodialysis patients.

A non-probability purposive sampling method was applied.

**2.2.3. Study instruments and equipment**

The DxH 900 analyzer (Beckman Coulter) was used for complete blood count testing. The AU5800 and DxI 800 systems (Beckman Coulter) and Cobas 8000 (Roche) were used for biochemical tests. Serum Klotho was quantified using an automated ELISA system (Immunomat, 4-plate, Germany). Bone mineral density was measured using a DXA Hologic system.

**2.2.4. Study procedures**

*2.2.4.5. Definitions and measurement of study variables*

Clinical variables included height, weight, body mass index (BMI), blood pressure, 24-hour urine volume, medical history (diabetes mellitus, hypertension, stroke, myocardial infarction, smoking), family history of kidney disease, duration of CKD, and dialysis vintage.

Laboratory variables included serum urea and creatinine, mineral–bone parameters (calcium, phosphate, parathyroid hormone [PTH], and 25-hydroxyvitamin D [25(OH)D]), lipid profile (total cholesterol, triglycerides, LDL-C, HDL-C), serum albumin, complete blood count,

and the urinary albumin-to-creatinine ratio. Laboratory testing was conducted according to standardized procedures and met ISO 15189:2012 requirements at the Hematology and Biochemistry Departments of the hospital.

*2.2.4.6. Bone mineral density measurement and assessment*

Bone mineral density was measured using dual-energy X-ray absorptiometry (DXA) with a Hologic Discovery Wi system at the lumbar spine and proximal femur. Osteoporosis was assessed using the T-score. According to the World Health Organization, osteoporosis was diagnosed when T-score ≤ −2.5 SD, and osteopenia was defined as a T-score between −1.0 and −2.5 SD.

*2.2.4.7. Measurement of serum Klotho and interpretation*

Serum Klotho was measured using an enzyme-linked immunosorbent assay (ELISA) on the Immunomat system (Germany) with the Human soluble α-Klotho Assay Kit (IBL, Japan), with a sensitivity of 6.15 pg/mL, intra-assay coefficient of variation < 3.5%, and inter-assay coefficient of variation < 11.4%.

Venous blood samples were collected in tubes without anticoagulant, allowed to clot for 30 minutes, and centrifuged at 3,000 rpm at 4°C for 15 minutes. Serum was separated and stored at −40°C. Each sample was thawed only once and analyzed in a single batch to minimize technical variability. Serum Klotho concentrations were determined by interpolation from the standard curve (93.75–6,000 pg/mL).

**2.2.5. Statistical analysis:** Data were analyzed using SPSS version 22.0.

Categorical variables were presented as frequency and percentage, and continuous variables as mean ± SD or median. Statistical significance was set at $p < 0.05$.

Proportions were compared using the chi-square test or Fisher's exact test. Means were compared using the t-test or one-way ANOVA (for normally distributed data), and the Mann–Whitney U test or

Kruskal–Wallis test (for non-normally distributed data).

Pearson or Spearman correlation coefficients and univariable/multivariable regression models (linear or logistic) were used to analyze associated factors. Receiver operating characteristic (ROC) curve analysis was performed to evaluate diagnostic performance using the area under the curve (AUC).

### 2.3. RESEARCH ETHICS

The study was approved by the Ethics Committee in Biomedical Research of Hue University of Medicine and Pharmacy, Hue University (No. H2022/502) and Thong Nhat Hospital (No. 61/2022/BVTN-HĐYĐ).

The costs of serum Klotho testing and other study-related expenses were covered by the investigators to ensure participants' benefits.

### 2.4. STUDY FLOW DIAGRAM

## Chapter 3

## RESEARCH RESULTS

### 3.1. CHARACTERISTICS OF THE STUDY POPULATION

### 3.2. SERUM KLOTHO LEVELS IN PATIENTS WITH CHRONIC KIDNEY DISEASE

#### 3.2.1. Serum Klotho levels by study group and CKD stage

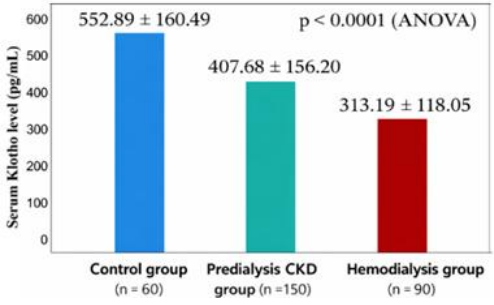


**Figure 3.2.** Serum Klotho Levels Across Study Groups

Klotho levels progressively decreased from the control group to the chronic kidney disease group and were lowest in the hemodialysis group.

**Table 3.17.** Serum Klotho Levels by CKD Stage

| Group | | Control (n=60)[a] | Stage 1 (n=30)[b] | Stage 2 (n=30)[c] | Stage 3 (n=30)[d] | Stage 4 (n=30)[e] | Stage 5 (n=30)[f] |
|---|---|---|---|---|---|---|---|
| Serum Klotho level (pg/mL) | Minimum | 313.84 | 225.52 | 176.25 | 196.53 | 127.50 | 116.25 |
| | Maximum | 1058.17 | 882.43 | 885.44 | 598.40 | 515.02 | 623.67 |
| | Mean ± SD | 552.89 ± 160.49 | 497.9 ± 151.32 | 490.4 ± 183.16 | 395.1 ± 116.12 | 335.44 ± 98.23 | 319.3 ± 128.97 |
| | p-value | $p^{a\&b} > 0.05$; $p^{a\&c} > 0.05$; $p^{a\&d} < 0.0001$; $p^{a\&e} < 0.0001$; $p^{a\&f} < 0.0001$; $p^{c\&d} < 0.05$ | | | | | |

Serum Klotho levels declined progressively with advancing CKD stages, with a statistically significant reduction beginning from stage 3.

### 3.2.2. Serum Klotho levels in hemodialysis patients

In the hemodialysis group, patients on dialysis for < 5 years had significantly higher mean Klotho levels (337.95 ± 117.09 pg/mL) compared with those on dialysis for ≥ 5 years (258.36 ± 102.17 pg/mL, $p < 0.05$; Mann–Whitney test) (Figure 3.4).

## 3.3. ASSOCIATIONS BETWEEN SERUM KLOTHO LEVELS AND ESTIMATED GLOMERULAR FILTRATION RATE, MINERAL–BONE PARAMETERS, AND BONE MINERAL DENSITY

### 3.3.1. Association between serum Klotho levels and estimated glomerular filtration rate in predialysis CKD patients

*3.3.1.1. Correlation between serum Klotho levels and renal function markers*

**Table 3.14.** Correlation between serum Klotho levels and renal function markers

| Variable | | Urea | Creatinine | eGFR |
|---|---|---|---|---|
| Klotho | r | −0.39 | −0.46 | 0.45 |
| | p-value | < 0.001 | < 0.001 | < 0.001 |

Serum Klotho levels showed a significant negative correlation with urea and creatinine, and a positive correlation with eGFR ($p < 0.001$).

*3.3.1.2. Diagnostic value of serum Klotho in identifying reduced estimated glomerular filtration rate*

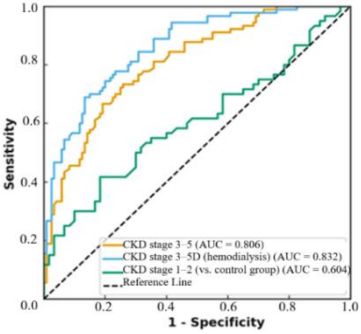


**Figure 3.6.** ROC Curve of Serum Klotho levels for identifying decreased eGFR.

**Table 3.26.** Diagnostic Performance of Serum Klotho in Identifying Reduced eGFR and CKD Stages.

| Model | AUC | p-value | 95% CI | Cutoff (pg/mL) | Youden Index | Se | Sp |
|---|---|---|---|---|---|---|---|
| Controls vs CKD G1–G2 | 0.604 | < 0.05 | 0.502–0.706 | 424.2 | 0.217 | 0.81 | 0.40 |
| CKD ≤ G2 vs CKD G3–G5 | 0.806 | < 0.01 | 0.747–0.864 | 390.8 | 0.475 | 0.81 | 0.67 |
| CKD ≤ G2 vs CKD G3-G5D | 0.832 | < 0.01 | 0.787–0.878 | 397.1 | 0.500 | 0.80 | 0.70 |

The AUC of serum Klotho levels increased across the models and was highest for the comparison between CKD stage ≤ 2 and CKD stages 3–5D, suggesting that Klotho has good discriminative ability in advanced-stage CKD.

**3.3.2. Association between serum Klotho levels and mineral–bone parameters**

*3.3.2.1. Comparison of serum Klotho levels according to mineral–bone abnormalities*

**Table 3.16.** Serum Klotho Levels According to Calcium–Phosphate Disorders in the CKD Group (n = 240)

| Parameter | Subgroup | n | % | Klotho (pg/mL) Mean ± SD | p-value |
|---|---|---|---|---|---|
| Corrected calcium (mmol/L) | Low (< 2.10) | 36 | 15% | 326.49 ±133.19 | > 0.05 |
| | Normal (2.10-2.54) | 186 | 77.5% | 379.31 ±149.09 | |
| | High (> 2.54) | 18 | 7.5% | 390.79 ±180.48 | |
| Serum phosphate (mmol/L) | Normal (≤ 1.78) | 190 | 79.2% | 389.38 ±152.50 | < 0.001 |
| | High (> 1.78) | 50 | 20.8% | 307.15 ±120.76 | |
| Ca × P ($mmol^2/L^2$) | Normal (< 4.4) | 205 | 85.4% | 382.71 ±153.39 | < 0.05 |
| | High (≥ 4.4) | 35 | 14.6% | 310.94 ±111.64 | |

Klotho levels did not differ across corrected calcium subgroups; however, they were significantly lower in patients with hyperphosphatemia and in those with an elevated calcium–phosphate product.

In the CKD group, serum Klotho levels were significantly higher in patients with PTH <150 pg/mL compared with those with PTH ≥150 pg/mL (431.66 ± 156.68 vs. 311.74 ± 112.79 pg/mL, $p < 0.001$). In contrast, in the hemodialysis group, no significant difference in Klotho levels was observed between patients with PTH <150 pg/mL and those with PTH ≥150 pg/mL (343.35 ± 141.08 vs. 308.55 ± 14.46 pg/mL, $p > 0.05$) (Table 3.17).

In the CKD group, mean serum Klotho levels were 379.09 ± 153.20 pg/mL in patients with 25(OH)D < 30 ng/mL and 363.30 ± 145.97 pg/mL in those with ≥ 30 ng/mL, with no statistically significant difference ($p > 0.05$) (Figure 3.8).

*3.3.2.2. Correlation between serum Klotho levels and mineral–bone parameters*

**Table 3.20.** Correlation between serum Klotho levels and mineral–bone parameters by patient group

| Group | | Ca | P | Ca×P | PTH | 25(OH)D |
|---|---|---|---|---|---|---|
| Predialysis CKD | r | 0.08 | −0.13 | −0.11 | **−0.37** | 0.004 |
| | p-value | 0.35 | 0.12 | 0.17 | **< 0.001** | 0.96 |
| Hemodialysis | r | −0.17 | −0.09 | −0.12 | −0.17 | −0.04 |
| | p-value | 0.11 | 0.4 | 0.28 | 0.1 | 0.72 |

In predialysis CKD patients, Klotho levels showed a significant negative correlation with PTH ($p < 0.001$), but no significant association with other parameters. In the hemodialysis group, no significant correlations were observed.

### 3.3.3. Association between serum Klotho levels and bone mineral density

*3.3.3.1. Comparison of serum Klotho levels according to bone mineral density classification at measurement sites*

**Table 3.22**. Comparison of serum Klotho levels among patient groups based on bone mineral density at different measurement sites in the hemodialysis group

| Site | BMD category | n | % | Klotho (pg/mL) Mean ± SD | p-value |
|---|---|---|---|---|---|
| Lumbar spine (L1–L4) | Osteoporosis | 18 | 20.0 | 269.50 ± 106.54 | > 0.05 |
| | Osteopenia | 31 | 34.4 | 328.78 ± 142.62 | |
| | Normal | 41 | 45.6 | 320.58 ± 99.12 | |
| Femoral neck | Osteoporosis | 33 | 36.7 | 292.85 ± 133.03 | > 0.05 |
| | Osteopenia | 40 | 44.4 | 321.19 ± 110.37 | |
| | Normal | 17 | 18.9 | 333.85 ± 104.72 | |
| Total Hip | Osteoporosis | 19 | 21.1 | 255.99 ± 110.28 | **< 0.05** |
| | Osteopenia | 30 | 33.3 | 346.57 ± 117.96 | |
| | Normal | 41 | 45.6 | 315.27 ±114.24 | |
| Total body | Osteoporosis | 36 | 40.0 | 297.91 ± 132.46 | > 0.05 |
| | Osteopenia | 40 | 44.4 | 319.45 ± 106.18 | |
| | Normal | 14 | 15.6 | 334.59 ± 114.40 | |

Mean serum Klotho levels did not differ among the osteoporosis, osteopenia, and normal groups at the lumbar spine, femoral neck, or total body ($p > 0.05$). However, at the total hip, Klotho levels differed significantly ($p < 0.05$), with the lowest values observed in the osteoporosis group.

*3.3.3.2. Correlation between serum Klotho levels and bone mineral density*

**Table 3.23.** Correlation between serum Klotho levels and bone mineral density and T-scores at measurement sites by patient group

| Group / Characteristics | | CKD stage 3–5 | | Hemodialysis | |
|---|---|---|---|---|---|
| Measurement site | Variable | r | p-value | r | p-value |
| Lumbar spine | BMD | 0.03 | 0.77 | 0.13 | 0.24 |
| | T-score | 0.01 | 0.89 | 0.13 | 0.24 |
| Femoral neck | BMD | 0.004 | 0.97 | 0.12 | 0.25 |
| | T-score | 0.02 | 0.86 | 0.12 | 0.27 |
| Total hip | BMD | 0 | 0.96 | 0.12 | 0.27 |
| | T-score | 0.02 | 0.83 | 0.11 | 0.31 |

No significant correlation was found between serum Klotho levels and BMD or T-scores at any site in both CKD stage 3–5 and hemodialysis groups ($p > 0.05$).

*3.3.3.3. Regression analysis of the association between serum Klotho levels and bone mineral density*

Univariate logistic regression analysis showed no statistically significant association between serum Klotho levels and osteoporosis in both the CKD stage 3–5 and hemodialysis groups ($p > 0.05$). Odds ratios (ORs) close to 1 indicate that Klotho does not have a significant impact on the risk of osteoporosis (Table 3.25).

**3.3.4. Independent factors associated with serum Klotho levels**

*3.3.4.1. Regression analysis of factors associated with Klotho levels in predialysis CKD patients*

Univariate linear regression analysis in the predialysis CKD group identified seven factors significantly associated with serum Klotho levels ($p < 0.05$). Among these, eGFR showed the strongest correlation

($R^2$ = 0.206), followed by creatinine, urea, PTH, hemoglobin, the number of mineral–bone disorders, and age, which showed the weakest correlation ($R^2$ = 0.037) (Table 3.26).

**Table 3.27.** Multivariate linear regression analysis of factors associated with serum Klotho levels in predialysis CKD patients

| Variable | Unstandardized B | Standardized Beta | t | p-value |
|---|---|---|---|---|
| Constant | 307.517 | - | 3.061 | 0.003 |
| eGFR (mL/min/1.73 m²) | 2.172 | 0.487 | 4.583 | < 0.001 |
| Hemoglobin (g/dL) | -1.357 | -0.027 | -0.188 | 0.851 |
| Age (years) | 0.171 | 0.014 | 0.202 | 0.840 |

eGFR, hemoglobin, and age together explained 20.7% of the variation in serum Klotho levels ($R^2$ = 0.207; $p < 0.0001$). In the multivariable regression model, eGFR was the strongest independent factor associated with serum Klotho (β = 0.487). Regression equation:

**Serum Klotho (pg/mL) = 2.172 × eGFR (mL/min/1.73 m²) – 1.357 × Hemoglobin (g/dL) + 0.171 × Age (years) + 307.517**

*3.3.4.2. Regression analysis of factors associated with serum Klotho levels in hemodialysis patients*

Univariate linear regression analysis in the hemodialysis group identified three factors significantly associated with serum Klotho levels ($p < 0.05$). Among these, hemoglobin showed the strongest correlation ($R^2$ = 0.115), followed by dialysis vintage ($R^2$ = 0.062) and duration since CKD diagnosis ($R^2$ = 0.048) (Table 3.28).

**Table 3.29.** Multivariate linear regression analysis of factors associated with serum Klotho levels in hemodialysis patients

| Variable | Unstandardized B | Standardized Beta | t | p-value |
|---|---|---|---|---|
| Constant | 238.123 | - | 1.478 | 0.143 |
| Dialysis vintage | -0.655 | -0.224 | -2.167 | 0.033 |
| Hemoglobin | 26.180 | 0.292 | 2.905 | 0.005 |
| Diastolic BP | -2.084 | -0.152 | -1.508 | 0.135 |

With R = 0.413 and $R^2$ = 0.170, the three variables - dialysis vintage,

hemoglobin, and diastolic blood pressure - explained 17% of the variation in serum Klotho levels. However, only hemoglobin ($\beta$ = 0.292; p = 0.005) and dialysis vintage ($\beta$ = –0.224; p = 0.033) remained independently associated with serum Klotho levels. Regression equation:

**Serum Klotho (pg/mL) = –0.655 × Dialysis vintage (months) + 26.180 × Hemoglobin (g/dL) – 2.084 × Diastolic blood pressure (mmHg) + 238.123**

# Chapter 4
# DISCUSSION

## 4.1. CHARACTERISTICS OF THE STUDY POPULATION

## 4.2. SERUM KLOTHO LEVELS IN PATIENTS WITH CHRONIC KIDNEY DISEASE

### 4.2.2. Serum Klotho levels across study groups

Serum Klotho levels decreased progressively from controls to CKD patients and were lowest in those receiving hemodialysis ($p < 0.05$), consistent with international findings. Variations in absolute values across studies may reflect differences in assay methods, population characteristics, and confounding factors such as inflammation, oxidative stress, uremic toxins, and treatment effects. In hemodialysis patients, Klotho levels were markedly reduced, consistent with reports by Yokoyama and others. This reduction may be influenced by dialysis vintage and modality, chronic inflammation, comorbidities, and therapeutic interventions. Further standardized and well-controlled studies are warranted to clarify the prognostic significance of Klotho in hemodialysis populations.

### 4.2.3. Serum Klotho levels according to CKD stages

This study showed that serum Klotho levels progressively declined with CKD progression, becoming significantly reduced from stage 3 onward ($p < 0.05$) and more pronounced in stages 4–5 ($p < 0.001$), suggesting that Klotho may reflect CKD severity. Similar trends have been reported by Shimamura, Pavik, Liu, and Rotondi, with some studies demonstrating a decline in Klotho as early as CKD stage 2 and suggesting its role as an early biomarker. However, absolute Klotho values vary across studies, which may be attributable to differences in

assay methods, commercial kits, and study populations. In addition, tubular injury, inflammation, and treatment-related factors may also influence circulating Klotho levels. Notably, some studies, such as that by Seiler, did not demonstrate a clear stage-dependent decline. In summary, serum Klotho appears to have potential as a biomarker reflecting CKD progression; however, further studies are required to clarify its diagnostic value for early CKD detection.

#### 4.2.4. Serum Klotho levels according to dialysis vintage (< 5 years vs ≥ 5 years)

Serum Klotho levels were lower in patients with dialysis vintage ≥60 months and were inversely correlated with dialysis vintage ($r = -0.29$; $p = 0.005$), suggesting a decline with prolonged hemodialysis. This contrasts with some previous reports and may reflect differences in populations or assay methods. Klotho levels are also influenced by residual kidney function, inflammation, and oxidative stress. Overall, dialysis vintage appears associated with reduced Klotho, supporting its potential role as a biomarker of dialysis-related disease burden in long-term maintenance hemodialysis patients.

### 4.3. ASSOCIATIONS BETWEEN SERUM KLOTHO LEVELS AND ESTIMATED GLOMERULAR FILTRATION RATE, MINERAL–BONE PARAMETERS, AND BONE MINERAL DENSITY

#### 4.3.1. Association between serum Klotho levels and estimated glomerular filtration rate in predialysis CKD patients

Serum Klotho levels declined progressively with CKD progression and showed a moderate positive correlation with eGFR ($r = 0.45$; $p < 0.001$), becoming significant from stage 3 onward. These findings are consistent with most previous studies and meta-analyses, although some reports have shown no clear association. This relationship likely reflects tubular injury and reduced renal Klotho production, along with the effects of inflammation and uremic toxins, suggesting that Klotho may serve as a marker of declining kidney function.

ROC analysis demonstrated good discrimination for advanced CKD (AUC = 0.832; $p < 0.01$), with a cutoff of 397.1 pg/mL (sensitivity 80.0%, specificity 70.0%), supporting its potential diagnostic value in

identifying CKD progression.

#### 4.3.2. Association between serum Klotho levels and mineral–bone parameters

Analysis of the associations between Klotho and mineral–bone parameters revealed:

**Corrected serum calcium:** Serum Klotho levels showed a slight, non-significant decline with decreasing corrected calcium, with no linear correlation observed in either the CKD or hemodialysis groups. ROC analysis indicated a modest discriminatory ability for hypocalcemia (AUC = 0.655; $p < 0.05$). Klotho is involved in calcium regulation through TRPV5 and the FGF23–vitamin D axis, but inconsistent findings across studies may reflect differences in patient populations, disease stages, and treatment factors. In hemodialysis patients, dialysis-related calcium fluctuations may further obscure this association.

**Serum phosphate**: Serum Klotho levels were significantly lower in patients with hyperphosphatemia ($p < 0.001$), but no significant correlations were observed in either group. ROC analysis showed limited discriminatory ability for hyperphosphatemia (AUC = 0.654; $p < 0.01$), lower than that of PTH. This aligns with previous reports of a weak inverse relationship between Klotho and phosphate. Variability across studies may be related to differences in renal function, inflammation, and treatment strategies, while dialysis and phosphate binder use may attenuate this association in hemodialysis patients.

**Serum PTH:** Serum Klotho levels were inversely correlated with PTH in the CKD group ($r = -0.37$; $p < 0.001$), but this association was not significant in the maintenance hemodialysis group. These findings support the inverse regulatory relationship between Klotho and PTH through the Klotho–FGF23 axis. Variability among studies suggests that the Klotho–PTH relationship may be influenced by multiple factors, with CKD–MBD pathophysiology playing a central role. In addition, ROC analysis showed that serum Klotho discriminated elevated PTH levels (≥150 pg/mL) in patients with chronic kidney disease, with an AUC of 0.712 ($p < 0.01$), suggesting that Klotho may have supportive value in assessing parathyroid dysfunction in advanced

CKD.

**Serum 25(OH)D:** No significant association was observed between Klotho and serum 25(OH)D levels ($p > 0.05$). Although some interventional studies have suggested that active vitamin D may increase Klotho expression, available observational evidence remains insufficient to establish a consistent relationship.

#### 4.3.3. Association between serum Klotho levels and bone mineral density and osteoporosis

In the predialysis CKD group, serum Klotho was not associated with BMD at any site ($p > 0.05$). In the hemodialysis group, Klotho was lower in patients with osteoporosis at the total hip ($p = 0.030$), but this was not significant in regression analysis. These findings may reflect differences between cortical and trabecular bone metabolism and potential confounding in DXA measurements (e.g., degenerative changes, calcification), along with limited sample size.

Previous studies have reported inconsistent results, with some showing no association and others suggesting a link between lower Klotho and reduced BMD or higher osteoporosis risk. This variability may relate to differences in populations, disease stages, and treatments.

Overall, Klotho was not clearly associated with BMD in this study, although lower levels in hemodialysis patients with osteoporosis suggest a potential role in CKD–MBD. Further longitudinal studies are needed to clarify its clinical relevance.

#### 4.3.4. Independent factors associated with serum Klotho levels: multivariable linear regression analysis

Linear regression analysis showed that eGFR was the strongest independent factor associated with serum Klotho levels in the predialysis CKD group (model $R^2 = 0.207$), whereas hemoglobin and dialysis vintage were the main determinants in the maintenance hemodialysis group (model $R^2 = 0.170$). International studies have also reported that eGFR, hemoglobin, phosphate, or age may be associated with serum Klotho levels; however, findings remain inconsistent. These results may reflect differences in Klotho regulation across CKD stages. Overall, Klotho has potential as a biomarker for assessing CKD

progression and mineral–metabolic homeostasis in maintenance hemodialysis patients.

In summary, this study demonstrated that serum Klotho levels progressively declined from the control group to CKD patients and were lowest in the maintenance hemodialysis group. Serum Klotho showed a clear positive correlation with eGFR, and ROC analysis indicated good discriminatory performance for advanced CKD (stages 3A–5D). Among maintenance hemodialysis patients, Klotho levels were significantly lower in those with osteoporosis at the hip region. In the predialysis CKD group, Klotho was inversely correlated with PTH; however, no significant associations were observed between Klotho and serum calcium, phosphate, or 25(OH)D in either the CKD or hemodialysis groups. With an adequate sample size, well-defined CKD stratification, an appropriate control group, and standardized laboratory procedures, our findings are consistent with international evidence and provide additional data in Vietnamese patients. Overall, Klotho appears to be a promising biomarker reflecting kidney function decline and CKD–MBD; however, further large-scale and longitudinal studies are warranted to confirm its clinical utility.

## CONCLUSION

Based on the assessment of serum Klotho levels and their associations with estimated glomerular filtration rate, selected mineral–bone parameters, and bone mineral density in 300 participants at Thong Nhat Hospital (from October 2022 to June 2024), including 60 healthy controls and 240 patients with chronic kidney disease (150 patients with predialysis CKD stages 1–5 and 90 patients receiving maintenance hemodialysis), we drew the following conclusions:

**1. Serum Klotho levels in chronic kidney disease**

Serum Klotho levels were markedly decreased in patients with chronic kidney disease and tended to decline with disease progression, particularly among maintenance hemodialysis patients. The main findings were as follows:

- Serum Klotho levels progressively decreased from the control group (552.89 ± 160.49 pg/mL) to the predialysis CKD stages 1–5 group (407.68 ± 156.20 pg/mL) and were lowest in the maintenance

hemodialysis group (313.19 ± 118.05 pg/mL), with a statistically significant difference ($p < 0.0001$).

- The mean serum Klotho levels across CKD stages 1, 2, 3, 4, and 5 were 497.95 ± 151.32, 490.46 ± 183.16, 395.17 ± 116.12, 335.44 ± 98.23, and 319.38 ± 128.97 pg/mL, respectively, demonstrating a decreasing trend with advancing CKD stage. This reduction became significant from stage 3 onward compared with the control group, as well as compared with stages 1 and 2.
- Among maintenance hemodialysis patients, those with dialysis vintage ≥ 60 months had a significantly lower mean serum Klotho level (258.36 ± 102.17 pg/mL) than those with dialysis vintage < 60 months (337.95 ± 117.09 pg/mL).

**2. Associations Between Serum Klotho Levels and Estimated Glomerular Filtration Rate, Mineral–Bone Parameters, and Bone Mineral Density**

**2.1. Association between serum Klotho levels and estimated glomerular filtration rate in predialysis CKD patients**

Serum Klotho levels were positively correlated with eGFR ($r = 0.45$; $p < 0.001$) and hemoglobin ($r = 0.31$; $p < 0.001$), and negatively correlated with urea ($r = −0.39$; $p < 0.001$) and creatinine ($r = −0.46$; $p < 0.001$).

ROC analysis showed that serum Klotho had good discriminatory ability for reduced eGFR (eGFR < 60 mL/min/1.73 m²; corresponding to CKD stages 3–5D), with an AUC of 0.832 (95% CI: 0.787–0.878), sensitivity of 80%, and specificity of 70% ($p < 0.01$).

In multivariable regression analysis, eGFR was the strongest independent factor associated with serum Klotho levels. The regression equation was:

**Klotho (pg/mL) = 2.172 × eGFR (mL/min/1.73 m²) − 1.357 × hemoglobin (g/dL) + 0.171 × age (years) + 307.517**

The model was statistically significant ($p < 0.001$), with an $R^2$ of 0.207, explaining approximately 20.7% of the variance in serum Klotho levels.

**2.2. Association between serum Klotho levels and mineral–bone parameters and bone mineral density**

*a. Association between serum Klotho levels and mineral–bone*

*parameters*

In the predialysis CKD group, serum Klotho levels were inversely correlated with PTH ($r = -0.37$; $p < 0.001$), but showed no significant associations with serum calcium, phosphate, or 25(OH)D ($p > 0.05$).

In the maintenance hemodialysis group, serum Klotho levels were inversely correlated with dialysis vintage ($r = -0.29$; $p = 0.005$) and positively correlated with hemoglobin ($r = 0.34$; $p < 0.001$). No significant correlations were observed between Klotho and calcium, phosphate, 25(OH)D, or PTH.

Multivariable regression analysis identified dialysis vintage and hemoglobin as the two main determinants of serum Klotho levels in maintenance hemodialysis patients. The full regression equation was: **Klotho (pg/mL) = −0.655 × dialysis vintage (months) + 26.180 × hemoglobin (g/dL) − 2.084 × diastolic blood pressure (mmHg) + 238.123**

The model was statistically significant ($p < 0.001$), with an $R^2$ of 0.170, explaining approximately 17% of the variance in serum Klotho levels.

*b. Association Between Serum Klotho Levels and Bone Mineral Density*

Among maintenance hemodialysis patients, serum Klotho levels were significantly lower in those with osteoporosis at the total hip site (255.99 ± 110.28 pg/mL) compared with those with osteopenia or normal bone mineral density (315.27 ± 114.24 pg/mL; $p < 0.05$). However, no significant linear correlations were observed between Klotho and bone mineral density at other skeletal sites.

## RECOMMENDATIONS

### 1. Application of Klotho in Clinical Practice

Serum Klotho levels decline with CKD progression and dialysis vintage, with a marked reduction from stage 3 onward. Klotho may serve as an adjunct biomarker in selected populations, particularly in patients with CKD stage ≥ 3, CKD–MBD, or long-term hemodialysis.

ROC analysis showed good discrimination for reduced kidney function (eGFR <60 mL/min/1.73 m²), supporting its potential role in assessing renal function decline in high-risk patients.

**2. Early Detection and Intervention for CKD–MBD**

Serum Klotho may serve as an adjunct biomarker alongside conventional parameters (calcium, phosphate, PTH, 25-hydroxyvitamin D, and BMD) in the assessment of CKD–MBD.

Given the cross-sectional design, causality cannot be established. However, optimizing factors associated with low Klotho such as hyperphosphatemia, elevated PTH, and anemia—may help improve CKD–MBD and reduce bone–vascular complications.

**3. Directions for Future Research**

Longitudinal studies are needed to assess changes in serum Klotho during CKD progression and in response to treatment.

Incorporating additional biomarkers (e.g., FGF23, sclerostin, and $25(OH)_2D_3$) may help develop comprehensive models for CKD–MBD and define clinically relevant Klotho thresholds across CKD stages.

## RELATED SCIENTIFIC PUBLICATIONS

1. Nguyen Minh Quan, Vo Tam. Study on Serum Klotho Levels and Their Associations with Mineral–Bone Disorders in Hemodialysis Patients. Hue Journal of Medicine and Pharmacy – Hue University of Medicine and Pharmacy. Special Issue for the Urology–Nephrology Conference; August 2024: pp. 598–607. ISSN: 3030-4318; eISSN: 3030-4326.
2. Nguyen Minh Quan, Vo Tam. Study on Serum Klotho Levels and Their Associations with Estimated Glomerular Filtration Rate and Selected Biochemical Parameters in Patients with Chronic Kidney Disease. Hue Journal of Medicine and Pharmacy – Hue University of Medicine and Pharmacy. 2025; Volume 15, Issue 1: pp. 152–160. ISSN: 3030-4318; eISSN: 3030-4326. DOI: 10.34071/jmp.2025.1.21.
3. Nguyen Minh Quan, Vo Tam. Assessment of osteoporosis risk and factors influencing bone mineral density in patients with advanced chronic kidney disease and those undergoing maintenance hemodialysis. *Vietnam Journal of Community Medicine*. 2025;66(Special Issue 19):260-267. ISSN: 2354-0613. doi:10.52163/yhc.v66iCD19.3713.