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

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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  $\geq 20$  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 from stages 1 to 5, including those undergoing maintenance hemodialysis.*

*2. To investigate the relationship between serum Klotho levels and glomerular filtration rate in non-dialysis CKD patients, and to assess their associations with mineral–bone parameters (serum calcium, phosphate, parathyroid hormone, 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 148 pages: Introduction (3 pages), Literature Review (39 pages), Subjects and Methods (29 pages), Results (35 pages), Discussion (39 pages), Conclusion (2 pages), and Recommendations (1 page). It includes 56 tables, 10 figures, 1 diagram, 15 charts, and 172 references (12 Vietnamese, 160 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  $\leq 60$  mL/min/1.73 m<sup>2</sup>. 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 m<sup>2</sup>.

#### *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–mineral and bone disorder (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 (CKD–MBD)**

##### *1.1.2.1. Definition of CKD-MBD*

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 (PTH), 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 CKD*

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 m<sup>2</sup>. 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 m<sup>2</sup>, 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)<sub>2</sub>D<sub>3</sub> 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 (K11

and K12), can be cleaved by the proteases ADAM10 and ADAM17, generating soluble Klotho or K11/K12 fragments that are released into the circulation. Soluble Klotho is detectable in blood, cerebrospinal fluid, and urine.

#### *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 1 $\alpha$ -hydroxylase activity, leading to decreased synthesis of 1,25(OH)<sub>2</sub>D<sub>3</sub>. 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<sup>+</sup>–Cl<sup>–</sup> 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.

#### **1.2.2. Association between serum Klotho levels and glomerular filtration rate and mineral–bone metabolism disorders**

#### **1.3. CURRENT RESEARCH STATUS**

##### **1.3.1. International Studies**

Studies in non-dialysis CKD populations 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  $\pm$  1810.2 pg/mL in healthy controls to 460.2  $\pm$  222.8 pg/mL in patients with CKD stage 5. Similarly, Rotondi et al. (2015) found significantly lower Klotho levels in CKD patients (519  $\pm$  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  $379.93 \pm 143.66$  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 ( $119.10 \pm 47.29$  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  $\geq 18$  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 m<sup>2</sup> 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.

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

According to Rotondi, the mean serum Klotho level in CKD patients was  $519 \pm 183$  pg/mL, which was lower than that in controls ( $845 \pm 330$  pg/mL). With  $\alpha = 0.05$  and power = 95% ( $\beta = 0.05$ ), the constant C = 13.0. The calculated sample size was approximately 27 participants per group. This study included 300 participants: 60 healthy controls, 150 non-dialysis 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  $\leq -2.5$  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  $\alpha$ -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  $4^{\circ}\text{C}$  for 15 minutes. Serum was separated and stored at  $-40^{\circ}\text{C}$ . 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  $\pm$  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-HĐYD).

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

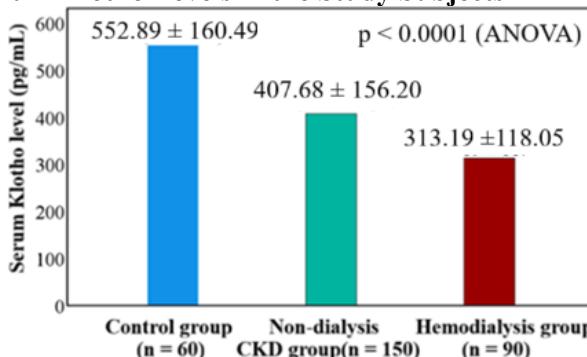
#### Chapter 3

### 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 in the Study Subjects



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

**Observation:** Serum 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) <sup>a</sup>	Stage 1 (n=30) <sup>b</sup>	Stage 2 (n=30) <sup>c</sup>	Stage 3 (n=30) <sup>d</sup>	Stage 4 (n=30) <sup>e</sup>	Stage 5 (n=30) <sup>f</sup>
Serum Klotho level (pg/mL)	Minimum	313.84	225.52	176.25	196.53	127.50
	Maximum	1058.17	882.43	885.44	598.40	515.02
	Mean±SD	552.89± 160.49	497.9± 151.32	490.4± 183.16	395.1± 116.12	335.44± 98.23
p-value	p <sup>a&amp;b</sup> >0.05; p <sup>a&amp;c</sup> >0.05; p <sup>a&amp;d</sup> <0.0001; p <sup>a&amp;e</sup> <0.0001; p <sup>a&amp;f</sup> <0.0001; p <sup>c&amp;d</sup> <0.05					

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

In the hemodialysis group, patients on dialysis for < 5 years had significantly higher mean serum 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 3.3).

**Table 3.20.** 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	
Calcium × Phosphate product (mmol <sup>2</sup> /L <sup>2</sup> )	Normal (< 4.4)	205	85.4%	382.71 ±153.39	< 0.05
	High	35	14.6%	310.94	

	( $\geq$ 4.4)			$\pm 111.64$	
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**Observation:** Serum 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, mean serum Klotho levels were  $379.09 \pm 153.20$  pg/mL in patients with  $25(\text{OH})\text{D} < 30$  ng/mL and  $363.30 \pm 145.97$  pg/mL in those with  $\geq 30$  ng/mL, with no statistically significant difference ( $p > 0.05$ ) (Figure 3.5).

**Table 3.21.** Serum Klotho Levels According to PTH Categories Across Study Group

		Klotho (pg/mL)	Control <sup>1</sup> (n = 60)	Non-dialysis CKD <sup>2</sup> (n = 150)	Hemodialysis <sup>3</sup> (n = 90)
PTH (pg/mL)	<150 <sup>(a)</sup> (n=192)	Mean $\pm$ SD	552.89 $\pm$ 160.49	431.66 $\pm$ 156.68	343.35 $\pm$ 141.08
		p-value	$p^{1&2} < 0.0001$ ; $p^{1&3} < 0.0001$ ; $p^{2&3} > 0.05$		
		n (%)	60(100)	120 (80)	12 (13.3)
	$\geq 150$ <sup>(b)</sup> (n=108)	Mean $\pm$ SD		311.74 $\pm$ 112.79	308.55 $\pm$ 14.46
		p-value	$p^{2&3} > 0.05$		
		n (%)		30 (20)	78 (86.7)
			$p^{(a)&(b)} < 0.001$		$p^{(a)&(b)} > 0.05$

**Observation:** In the CKD group, serum Klotho levels were significantly higher in patients with PTH  $< 150$  pg/mL, whereas no difference was observed between PTH categories in the hemodialysis group.

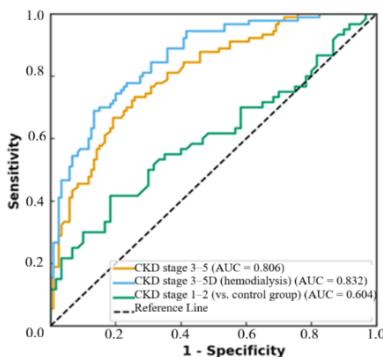
**Table 3.25.** Serum Klotho Levels According to Bone Mineral Density Categories at Different Measurement Sites in the Hemodialysis Group

Site	BMD category	n	%	Klotho (pg/mL) Mean $\pm$ SD	p-value
Lumbar spine (L1–L4)	Osteoporosis	18	20.0	$269.50 \pm 106.54$	>0.05
	Osteopenia	31	34.4	$328.78 \pm 142.62$	
	Normal	41	45.6	$320.58 \pm 99.12$	
Femoral neck	Osteoporosis	33	36.7	$292.85 \pm 133.03$	>0.05
	Osteopenia	40	44.4	$321.19 \pm 110.37$	

	Normal	17	18.9	$333.85 \pm 104.72$	
Total Hip	Osteoporosis	19	21.1	$255.99 \pm 110.28$	<0.05
	Osteopenia	30	33.3	$346.57 \pm 117.96$	
	Normal	41	45.6	$315.27 \pm 114.24$	
Total body	Osteoporosis	36	40.0	$297.91 \pm 132.46$	>0.05
	Osteopenia	40	44.4	$319.45 \pm 106.18$	
	Normal	14	15.6	$334.59 \pm 114.40$	

**Observation:** 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.

### 3.2.2. ROC Curves of Serum Klotho for Identifying CKD Stages and Mineral–Bone Metabolism Disorders



**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	0.400
CKD $\leq$ G2 vs CKD G3–G5	0.806	<0.01	0.747–0.864	390.8	0.475	0.808	0.667
CKD $\leq$ G2 vs	0.832	<0.01	0.787–	397.1	0.500	0.800	0.700

CKD G3–G5D			0.878				
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**Observation:** The AUC of serum Klotho levels increased across the models and was highest for the comparison between CKD stage  $\leq 2$  and CKD stages 3–5D, suggesting that Klotho has good discriminative ability in advanced-stage CKD.

### 3.3. ASSOCIATION BETWEEN SERUM KLOTHO LEVELS AND EGFR, MINERAL–BONE PARAMETERS, BONE MINERAL DENSITY, AND OTHER FACTORS IN PATIENTS WITH CKD

#### 3.3.1. Correlations Between Klotho and Clinical–Laboratory Parameters in the Non-Dialysis CKD Group

**Table 3.28.** Correlation Between Serum Klotho and Laboratory Parameters in the Non-Dialysis CKD Group

Parameter		Klotho	Ca <sub>corr</sub>	Phosphate	Ca x P	PTH	25(OH)D
Hemoglobin	r	0.31	0.21	-0.44	-0.38	-0.58	0.14
	p	<0.001	0.01	<0.001	<0.001	<0.001	0.08
Ure	r	-0.39	-0.22	0.5	0.45	0.7	-0.14
	p	<0.001	0.007	<0.001	<0.001	<0.001	0.10
Creatinin	r	-0.46	-0.19	0.49	0.44	0.74	-0.11
	p	<0.001	0.20	<0.001	<0.001	<0.001	0.18
MLCT	r	0.45	0.23	-0.44	-0.39	-0.61	0.02
	p	<0.001	0.004	<0.001	<0.001	<0.001	0.78

**Observation:** Serum Klotho levels showed a positive correlation with eGFR and hemoglobin, and an inverse correlation with urea and creatinine in the CKD group.

In patients with CKD, Klotho was negatively correlated with PTH ( $r = -0.37$ ;  $p < 0.001$ ), whereas no significant correlations were observed with corrected calcium ( $r = 0.08$ ;  $p = 0.35$ ), phosphate ( $r = -0.13$ ;  $p = 0.12$ ), calcium–phosphate product ( $r = -0.11$ ;  $p = 0.17$ ), or 25(OH)D ( $r = 0.004$ ;  $p = 0.96$ ) (Table 3.29).

**Table 3.30.** Correlation Analysis Between Serum Klotho Levels and Bone Mineral Density and T-scores at Three Measurement Sites in the CKD G3–G5 Group (n = 90)

Site	Variable	r	p-value
Lumbar spine (L1–L4)	BMD	0.03	0.77
	T-score	0.01	0.89
Femoral neck	BMD	0.004	0.97
	T-score	0.02	0.86
Total hip	BMD	-0.00	0.96
	T-score	0.02	0.83

**Observation:** No significant correlations were observed between serum Klotho levels and either bone mineral density or T-scores at the femoral neck, total hip, or lumbar spine (p > 0.05).

Univariable linear regression analysis in the non-dialysis CKD group identified seven factors significantly associated with serum Klotho levels (p < 0.05), with eGFR showing the strongest association ( $R^2 = 0.206$ ), followed by creatinine, urea, PTH, hemoglobin, the number of mineral–bone abnormalities, and age ( $R^2 = 0.037$ ), which had the weakest association (Table 3.31).

**Table 3.32.** Multivariable Linear Regression Analysis of Factors Associated With Serum Klotho Levels in the CKD Group

Variable	Unstandardized B	Standardized Beta	t	p-value
Constant	307.517	-	3.061	0.003
eGFR (mL/min/1.73 m <sup>2</sup> )	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

**Observation:** 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 ( $\beta = 0.487$ ). Regression equation:

$$\text{Serum Klotho (pg/mL)} = 2.172 \times \text{eGFR (mL/min/1.73 m}^2\text{)} - 1.357 \times \text{Hemoglobin (g/dL)} + 0.171 \times \text{Age (years)} + 307.517$$

Univariable linear and logistic regression analyses showed no significant associations between Klotho and BMD, T-scores, or osteoporosis status in patients with advanced CKD ( $p > 0.05$ ). The correlation coefficients ( $R$ ), coefficients of determination ( $R^2$ ), and odds ratios ( $\approx 1$ ) were all low, indicating that Klotho did not meaningfully influence bone density or osteoporosis risk in this group (Tables 3.33 and 3.34).

### 3.2.2. Correlations Between Klotho and Clinical Parameters in the Hemodialysis Group

Serum Klotho levels demonstrated a negative correlation with dialysis vintage ( $r = -0.29$ ;  $p = 0.005$ ) (Table 3.35).

Klotho showed no significant correlations with mineral–bone parameters, including corrected calcium ( $r = -0.17$ ;  $p = 0.11$ ), phosphate ( $r = -0.09$ ;  $p = 0.40$ ), calcium–phosphate product ( $r = -0.12$ ;  $p = 0.28$ ), PTH ( $r = -0.17$ ;  $p = 0.10$ ), or 25(OH)D ( $r = -0.04$ ;  $p = 0.72$ ) (Table 3.37).

**Table 3.40.** Multivariable Linear Regression Analysis of Factors Associated With Serum Klotho in the Hemodialysis Group

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

**Observation:** 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:

$$\text{Serum Klotho (pg/mL)} = -0.655 \times \text{Dialysis vintage (months)} + 26.180 \times \text{Hemoglobin (g/dL)} - 2.084 \times \text{Diastolic blood pressure (mmHg)} + 238.123$$

Univariable linear regression analysis showed no significant associations between Klotho and bone mineral density or T-scores in the hemodialysis group ( $p > 0.05$ ).

## Chapter 4

### DISCUSSION

#### 4.1. CHARACTERISTICS OF THE STUDY POPULATION

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

##### 4.2.1. 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.

##### 4.2.2. 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. These findings are consistent with those reported by Shimamura, Pavik, Liu, and Rotondi, in which some authors observed a decline as early as stage 2 and proposed Klotho 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.

##### 4.2.3. Serum Klotho levels according to dialysis vintage (< 5

### **years vs $\geq$ 5 years)**

Serum Klotho levels were significantly lower in patients with a dialysis vintage of  $\geq$  60 months (5 years) compared with those with  $<$  60 months ( $p < 0.05$ ), and were inversely correlated with dialysis vintage ( $r = -0.29$ ;  $p = 0.005$ ), suggesting that prolonged hemodialysis exposure may contribute to reduced Klotho levels. In contrast to the study by Memmos, which did not report this association, the discrepancy may be attributed to differences in population characteristics and assay methods. Serum Klotho levels are also influenced by tubular injury, residual kidney function, chronic inflammation, and oxidative stress. Overall, our findings suggest that dialysis vintage is associated with decreased Klotho levels, and that low Klotho may serve as a potential marker for assessing disease progression in long-term maintenance hemodialysis patients.

### **4.3. ASSOCIATION BETWEEN SERUM KLOTHO LEVELS AND EGFR, MINERAL–BONE PARAMETERS, BONE MINERAL DENSITY, AND OTHER FACTORS IN PATIENTS WITH CKD**

#### **4.3.1. Association between Klotho and Glomerular Filtration Rate**

Our study demonstrated that serum Klotho levels progressively declined with CKD progression and showed a moderate positive correlation with eGFR ( $r = 0.45$ ;  $p < 0.001$ ). The reduction in Klotho became significant from CKD stage 3 onward and was more pronounced in stages 4–5 ( $p < 0.001$ ). These findings are consistent with several international studies, including those by Liu et al. ( $r = 0.593$ ;  $p < 0.001$ ) in patients with CKD stages 3–5, Pavik et al. ( $r^2 = 0.41$ ;  $p < 0.0001$ ) in patients with CKD stages 1–5, and Shimamura et al. ( $r = 0.441$ ;  $p < 0.0001$ ). In contrast, Seiler's study ( $p = 0.098$ ) did not demonstrate a clear association. A meta-analysis by Wang et al. (2018), including 1,457 patients from nine studies, reported a pooled correlation coefficient of 0.35 ( $p < 0.05$ ) between Klotho and eGFR, which was not significantly influenced by age, geographic region, or baseline eGFR. This association likely reflects tubular injury—given that the kidney, particularly tubular cells, is the

primary source of Klotho synthesis—as well as the effects of inflammation, fibrosis, and uremic toxins. Therefore, Klotho may serve as a biomarker reflecting declining kidney function, particularly from CKD stage 3 onward.

ROC analysis further supported this finding, showing that Klotho had good discriminatory ability for advanced CKD. In patients with CKD stages 3–5D (vs. CKD stage  $\leq 2$ ), the AUC was 0.832 (95% CI: 0.787–0.878;  $p < 0.01$ ). A cutoff value of 397.1 pg/mL yielded a sensitivity of 80.0% and a specificity of 70.0% (Youden index = 0.500), suggesting the potential diagnostic value of Klotho in identifying progressive kidney function decline.

#### **4.3.2. Association between Klotho and Mineral–Bone Parameters**

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

**Corrected serum calcium:** Serum Klotho levels showed a slight decrease as calcium levels declined; however, this difference was not statistically significant, and no significant linear correlation between Klotho and calcium was observed in either the CKD or maintenance hemodialysis groups. ROC analysis showed that serum Klotho had a moderate discriminatory ability for hypocalcemia (AUC = 0.655;  $p < 0.05$ ). Although Klotho plays a regulatory role in TRPV5 activity and the FGF23–vitamin D axis, previous studies have reported inconsistent results, which may be attributable to differences in study populations, disease stages, chronic inflammation, endocrine disturbances, and treatment-related factors (e.g., calcium supplementation and phosphate binders). In maintenance hemodialysis patients, fluctuations in calcium levels related to dialysis and concomitant therapies may obscure this association. Nevertheless, the findings still suggest a potential role of Klotho in calcium regulation within the CKD–MBD context.

**Serum phosphate:** Serum Klotho levels were significantly lower in patients with hyperphosphatemia ( $p < 0.001$ ); however, no significant linear correlations were observed in the CKD group ( $r = -0.13$ ;  $p = 0.12$ ) or the maintenance hemodialysis group ( $r = -0.09$ ;  $p = 0.40$ ). ROC analysis showed that serum Klotho had a moderate

discriminatory ability for hyperphosphatemia (AUC = 0.654;  $p < 0.01$ ), which was lower than that of PTH (AUC = 0.839;  $p < 0.001$ ). Meta-analyses have reported a weak inverse correlation between Klotho and phosphate, particularly in CKD populations, which is consistent with its physiological role in promoting renal phosphate excretion. Differences across studies may be influenced by variations in patient populations, the severity of kidney dysfunction, chronic inflammation, and treatment strategies. In maintenance hemodialysis patients, phosphate fluctuations related to dialysis and the use of phosphate binders may attenuate the observed association between Klotho and phosphate.

**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 ( $\geq 150$  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.

**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 Klotho and Bone Mineral Density and Osteoporosis**

In the non-dialysis CKD group, serum Klotho levels were not significantly associated with bone mineral density at any measured sites ( $p > 0.05$ ). In the maintenance hemodialysis group, serum Klotho levels were significantly lower in patients with osteoporosis at the total hip site ( $p = 0.030$ ); however, this association was not statistically significant in regression analysis. These findings may be

attributable to metabolic differences between cortical and trabecular bone in the context of CKD–MBD. In addition, lumbar spine BMD assessed by DXA in dialysis patients may be confounded by degenerative changes or calcification, leading to falsely elevated measurements, and the sample size of the osteoporosis subgroups was limited; therefore, these results should be further confirmed in studies with larger sample sizes.

Several studies, such as that by Chewcharat (2022), reported no significant relationship between Klotho and osteoporosis. In contrast, other studies (e.g., Zhang, Jiang, and Zheng) suggested that lower Klotho levels may be associated with reduced bone mineral density or an increased risk of osteoporosis at specific skeletal sites and in certain populations. Overall, the evidence remains inconsistent, and further studies are needed to clarify this association. Although no statistically significant association was observed, this negative finding remains scientifically meaningful, suggesting that Klotho may not directly reflect changes in BMD at a single time point, particularly in a cross-sectional design and within the multifactorial context of CKD–MBD (including PTH, FGF23, active vitamin D, and other relevant clinical factors).

In summary, serum Klotho was not clearly associated with bone mineral density in CKD patients in our study, whereas the observed reduction in Klotho among hemodialysis patients with osteoporosis suggests a potential role of Klotho in CKD–MBD. Further multicenter longitudinal cohort studies with multivariable analyses are needed to determine the clinical value of Klotho in assessing bone health.

#### **4.3.4. Regression Analyses Between Klotho and Related Factors**

Linear regression analysis showed that eGFR was the strongest independent factor associated with serum Klotho levels in the non-dialysis 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 non-dialysis 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 selected clinical factors, laboratory parameters, mineral–bone disorders, 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 non-dialysis 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 ( $552.89 \pm 160.49$  pg/mL) to the non-dialysis CKD stages 1–5 group ( $407.68 \pm 156.20$  pg/mL) and were lowest in the maintenance hemodialysis group ( $313.19 \pm 118.05$  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  $497.95 \pm 151.32$ ,  $490.46 \pm 183.16$ ,  $395.17 \pm 116.12$ ,  $335.44 \pm 98.23$ , and  $319.38 \pm 128.97$  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  $\geq 60$  months had a significantly lower mean serum Klotho level ( $258.36 \pm 102.17$  pg/mL) than those with dialysis vintage  $< 60$  months ( $337.95 \pm 117.09$  pg/mL).

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

Serum Klotho levels were associated with kidney function, selected mineral–bone parameters, and bone mineral density in patients with chronic kidney disease, as demonstrated by the following results:

### **2.1. Association Between Serum Klotho Levels and Estimated Glomerular Filtration Rate (eGFR)**

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 m $^2$ ; 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:

$$\text{Klotho (pg/mL)} = 2.172 \times \text{eGFR (mL/min/1.73 m}^2\text{)} - 1.357 \times \text{hemoglobin (g/dL)} + 0.171 \times \text{age (years)} + 307.517$$

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

In the non-dialysis 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:

$$\text{Klotho (pg/mL)} = -0.655 \times \text{dialysis vintage (months)} + 26.180 \times \text{hemoglobin (g/dL)} - 2.084 \times \text{diastolic blood pressure (mmHg)} + 238.123$$

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.

## 2.3. 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 ( $255.99 \pm 110.28$  pg/mL) compared with those with osteopenia or normal bone mineral density ( $315.27 \pm 114.24$  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 progressively declined with the progression of chronic kidney disease and with longer dialysis vintage, with a marked decrease from CKD stage 3 onward. Therefore, serum Klotho testing may be considered as an additional biomarker in selected patient groups, including: patients with CKD from stage 3 or higher; those with mineral–bone disturbances (elevated PTH, hyperphosphatemia, and reduced bone mineral density); and maintenance hemodialysis patients, particularly those with longer dialysis vintage.

ROC analysis showed that serum Klotho had good discriminatory ability for reduced eGFR (eGFR  $< 60$  mL/min/1.73 m $^2$ ), suggesting its potential utility as an adjunct biomarker for assessing renal function decline in high-risk populations, as measured by ELISA in this study.

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

Serum Klotho testing may be used in combination with calcium, phosphate, PTH, 25(OH)D, and bone mineral density to facilitate early detection of CKD–mineral and bone disorder (CKD–MBD) and to monitor treatment response in patients with chronic kidney disease.

Although the cross-sectional design does not allow causal inference, priority should be given to controlling factors commonly associated with low Klotho levels, including hyperphosphatemia, elevated PTH, and anemia, in order to improve CKD–MBD status and reduce bone–vascular complications in CKD patients.

## **3. Directions for Future Research**

Longitudinal cohort studies are needed to evaluate changes in serum Klotho levels over the course of CKD progression and in response to treatment.

In addition, integrating other biomarkers such as FGF23, sclerostin, or active 25(OH)D may help develop a comprehensive prognostic model for CKD–MBD and determine clinically meaningful Klotho thresholds for each CKD stage.

## 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.