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Vitamin K2 Correlation with Cathepsin k and P1NP as markers of bone turnover in Osteoporosis in hemodialysis patients

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Abstract

Bone disease is highly prevalent in patients with chronic kidney disease on dialysis (CKD-5D). It can induce serious bone health problems, especially fragility fractures. Bone disease in patients with CKD-5D is the result of bone turnover abnormalities and the decrease of bone mineral density (BMD) Multiple factors are associated with reduced BMD and may affect bone health. Dual-energy X-ray absorptiometry (DXA) was performed to assess bone mineral density (BMD). Bone mass density. The remodeling process is tightly regulated, when the balance between bone resorption and bone formation shifts to net bone loss results in the development of osteoporosis in both men and women. In this study, we evaluate correlations between vitamin K2 with Cathepsin K as marker of bone resorption and P1N1 as marker of bone formation as well as correlation of vit K2, Cathepsin K and P1N1 with BMD measured by DEXA to access osteoporosis. We also assess the frequency and risk factors of osteoporosis and osteopenia in hemodialysis patients. Vit K2 was significantly lower in osteoporosis patients than osteopenia patients so vitamin K2 level in CKD patients on hemodialysis patients is important for bone health and has close relation to degree of osteoporosis.

Keywords: Osteoporosis, CTHK, P1NP, VitK2, DEXA.

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1. Introduction

Bone disease is highly prevalent in patients with chronic kidney disease on dialysis (CKD-5D) [1]. It can induce serious bone health problems, especially fragility fractures. Bone disease in patients with CKD-5D is the result of bone turnover abnormalities and the decrease of bone mineral density (BMD). Bone biopsy remains the gold standard for the diagnosis of bone turnover abnormalities. However, it is an invasive method and repetitive assessment of bone status cannot be possible. Measurements of serum bone turnover markers are not common practice in the management of CKD-5D patients [1]. Both bone resorption and bone formation markers in patients with CKD-5D may be up- or down-regulated by systemic hormones such as parathormone. Moreover, the assessment of bone mass in patients under dialysis is not yet codified. The measurement of BMD by dual-energy x-ray absorptiometry (DEXA) is recommended only in patients with a history of fractures. Multiple factors are associated with reduced BMD and may affect bone health [2]. Dual-energy X-ray absorptiometry (DXA) was performed to assess bone mineral density (BMD). Bone mass density, expressed in gm/cm^2, was measured at the lumbar spine, total hip, femoral neck, and total body. In the lack of diagnostic criteria for defining osteoporosis in men and premenopausal women, we used WHO criteria as a cutoff point. Osteopenia corresponded to T-score more than − 2.5 SD but less than − 1 SD. Osteoporosis was defined as T-score less than or equal to − 2.5 SD in at least one of these sites:

lumbar spine, femoral neck or total hip [3]. Osteoporosis was common among patients undergoing hemodialysis. 23% of patients have osteoporosis and 45% have osteopenia. Osteoporosis affected the hip more than the spine [4]. The field of bone turnover markers has developed considerably in past decades. Biochemical monitoring of bone metabolism depends upon measurement of enzymes and proteins released during bone formation and of degradation products produced during bone resorption. Various biochemical markers are now available that allow a specific and sensitive assessment of the rate of bone formation and bone resorption of the skeleton. [5,6] Although these markers are not currently recommended for use in the diagnosis of osteoporosis, they appear to be useful for the individual monitoring of osteoporotic patients treated with antiresorptive agents [7]. Procollagen type 1 contains N- and C-terminal extensions, which are removed by specific proteases during conversion of procollagen to collagen. The extensions are the C- and N-terminal propeptides of procollagen type 1 (P1CP and P1NP). Anti-P1NP antibodies are used to detect the trimeric structure of P1NP by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay. Measurement of P1NP appears to be a more sensitive marker of bone formation rate in osteoporosis. These assays are being developed for clinical use. Cathepsin K(CTSK) is a protease in osteoclasts crucial for bone resorption, making it a significant therapeutic target for osteoporosis. Cathepsin K is a potent enzyme that breaks down type I collagen (main component of the bone matrix) and other components of the bone matrix. This process is essential for osteoclasts to resorb bone tissue. It is the most abundant cysteine protease in osteoclasts, playing a key role in the process of bone removal from the body. Vitamin K is a fat-soluble vitamin. It was discovered in 1929 by the Danish scientist, Henrik Dam. Three vitamin K isoforms are known: vitamin K1 (phylloquinone), vitamin K2 (menaquinones), and vitamin K3 (menadione). Vitamin K1 is synthesized by plants and is the predominant form of vitamin K in the human diet. Vitamin K2 is a bacterial by-product and is mainly found in fermented products or in food with animal origins [8]. Vitamin K2 has different chemical variants (vitamers), abbreviated as MK-n, where 'n' specifies the number of isoprenyl units in the side chain. In humans, the most common MK is the short-chain MK-4, which is primarily produced endogenously via systemic conversion of K1 to MK-4. The long-chain forms of MKs, MK-7 through MK-10, are synthesized by intestinal bacteria in all mammals [9,10] Vitamin K2 plays a central role in calcium metabolism (the main mineral found in your bones and teeth) by activating the calcium-binding actions of two proteins — matrix GLA protein and osteocalcin, which help to build and maintain bones.

In recent years, there has been growing interest in promotion of bone health and inhibition of vascular calcification by vitamin K2. This vitamin regulates bone remodeling, an important process necessary to maintain adult bone. Bone remodeling involves removal of old or damaged bone by osteoclasts and its replacement by new bone formed by osteoblasts which are protected from apoptosis by Vitamin K2 [11]. The remodeling process is tightly regulated, when the balance between bone resorption and bone formation shifts to net bone loss results in the development of osteoporosis in both men and women.

In this study, we evaluate correlations between vitamin K2 with Cathepsin K as marker of bone resorption and P1N1 as marker of bone formation as well as correlation of vit K2, Cathepsin K and P1N1 with BMD measured by DEXA to access osteoporosis. We also to assess the frequency and risk factors of osteoporosis and osteopenia in hemodialysis patients.

2. Patients and Methods:

64 patients on regular hemodialysis 3 times per week in hemodialysis unit in Theodor Bilharz Research Institute TBRI, National Research Center Ethics Committee approved research protocol (FWA 00014747), Registration No.: 20 126 . All participants gave written permission.

Age of patients between 40 -60 years old
All patients have undergone:
1) full history and clinical examination

2) Assessment of vitamin K2 using ELISA (Enzyme Linked Immunosorbent Assay) technique

3) Assessment of Cathepsin K To use Magic Red, add the substrate directly to the cell culture media, incubate, and analyze. Because it is a cell permeant, it easily penetrates the cell membrane and the membranes of the internal cellular organelles – no lysis or permeabilization steps are required. If cathepsin enzymes are active, the Magic Red substrate is cleaved and the cresyl violet fluorophore will become fluorescent upon excitation. As enzyme activity progresses and more Magic Red substrate is cleaved, the signal will intensify, allowing researchers to watch the color develop over time. Samples can be analyzed by fluorescence microscopy or with a fluorescence plate reader. Hoechst 33342 and Acridine Orange are included in the kit to detect nuclear morphology and lysosomal organelle structure, respectively.

4) Assessment of P1N1 level: The P1N1 measurement refers to the peak-to-peak amplitude difference and latency (timing) of the P1 (first positive) and N1 (first negative) waves, which are components of sensory evoked potentials (EPs) such as cortical auditory evoked potentials (CAEPs), visual evoked potentials (VEPs), and vestibular evoked myogenic potentials (VEMPs).

5) Dual-energy X-ray absorptiometry (DXA) was performed to assess bone mineral density (BMD). Bone mass density, expressed in gm/cm^2, was measured at the lumbar spine, total hip, femoral neck, and total body. Because of lack of diagnostic criteria for defining osteoporosis in men and premenopausal women, we used WHO criteria as a cutoff point. Osteopenia corresponded to T-score more than − 2.5 SD but less than − 1 SD. Osteoporosis was defined as T-score less than or equal to − 2.5 SD in at least one of these sites: lumbar spine, femoral neck or total hip.

2.1. Statistical analysis

Statistical analysis was done by SPSS v27 (IBM Inc., Chicago, IL, USA). Shapiro-Wilks test and histograms were used to evaluate the normality of the distribution of data.

Quantitative parametric data were presented as mean and standard deviation (SD). Quantitative non-parametric data were presented as median and interquartile range (IQR) and were analyzed by Mann Whitney-test. Correlation between various variables was done using Pearson moment correlation equation for linear relation of normally distributed variables and Spearman rank correlation equation for non-normal variables/non-linear monotonic relation.

3. Results and discussion

3.1. Results

The mean value (± SD) of age was 49.4(± 9.55) years. Regarding bone metabolism markers, the median (IQR) of vit K2 was 0.18 (0.08 - 1.95) ng/ml, the median (IQR) of CTSK was 16.85 (10.45 - 25.18) pg/ml and the median (IQR) of P1NP was 29 (19.75 - 44.25) mcg/L.

3.2. Discussion

Bone disease is very common in patients with chronic kidney disease on dialysis (CKD-5D) [1]. It can induce serious bone health problems, especially fragility fractures, so it is essentially important to study factors affecting bone health in these patients. In our research we study correlation between bone turnover markers which they are Cathepsin K (as marker of bone resorption) and P1NP (as marker of bone formation) and degree of osteoporosis measured by T-Score index of DEXA scan finding that there in significantly negative correlation between Cathepsin-K level and degree of osteoporosis (presented by DEXA T-Score) meaning that with increasing level of Cathepsin -K, DEXA T- Score is decreased and degree of osteoporosis is increased. This finding supports what has done in 2017 as P1NP and CTX were flagged for standardization by the International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine (IOF and IFCCLM), 2017 to find that BTMs could be used to predict the fracture risk prediction and monitor treatment response [12]. But these BTMs are not widely used in clinical practice. This explains the need to access bone health and degree of osteoporosis using DEXA monitoring

The data on standardized reference intervals of PINP in Koreans are available. Further research for CTX assay in Koreans is needed. The standardized patient preparation and sample handling procedures is important to decrease analytical variability. In the patients with CKD, use of BTMs is limited. When used in combination with PTH, BSALP can predict adynamic bone disease. This review supports standardization and clinical use in the management of patients of osteoporosis. Interestingly, there is also substantial evidence from controlled studies that K2 may provide major benefits for bone health as a 2022 meta-analysis trusted Source of 16 studies in 6,425 postmenopausal women found that those taking vitamin K2 supplements had a positive effect on bone mineralization and increased bone strength [13]. These findings agreed with our results of positive correlation between vit K2 and DEXA -T-score (r=0.271 and P value = 0.03). This positive correlation means that with increasing level of vit K2, DEXA T- Score is increased and degree of osteoporosis is decreased. However, a 2023 review of trials conducted in the past decade shows conflicting results [14] A meta-analysis of 13 studies of MK-4 at 45 mg day^{-1} was published in 200, It revealed that MK-4 has a protective effect on BMD and reduced risk of hip, vertebral, and non-vertebral fractures [15]

In our study Vit K2 was significantly lower in osteoporosis patients than osteopenia patients (P value=0.001) and there was a positive correlation between vit K2 and DEXA -T-score. These results support needs to vit K2 in protection and prevent osteoporosis in hemodialysis patients. This is different than be found in other studies revealed that combining vitamin K2 with vitamin D3 [16] or bisphosphonates [17] showed an additional protective effect on osteoporosis *vs* vitamin K2 treatment alone, meaning that not all vitamin K2 studies showed positive effects.

Table 1: Age and bone metabolism markers of the patients studied

	(n=64)
Age (years)	49.4 ± 9.55
Vit K2 (ng/ml)	0.18 (0.08 - 1.95)
CTSK (pg/ml)	16.85 (10.45 - 25.18)
P1NP (mcg/L)	29 (19.75 - 44.25)

Data presented as mean ± SD or median (IQR).

CTSK: Cathepsin K, P1NP: Procollagen type 1 N-terminal propeptide.

The median (IQR) of Dexa -T-score was -1.7 (-2.23 - -1). Regarding dexa T-Score, 52 (81.25%) patients had osteopenia, and 12 (18.75%) patients had osteoporosis.

Table 2: Dexa -T-score and distribution of osteoporosis and osteopenia based on Dexa T-score of the studied patients

		(n=64)
Dexa -T-score		-1.7 (-2.23 - -1)
Dexa T-Score	**Osteopenia**	52 (81.25%)
	Osteoporosis	12 (18.75%)

Data presented as median (IQR) or number (%).

Vit K2 was significantly lower in osteoporosis patients than osteopenia patients (P value=0.001).

Table 3: Relation between Vit K2 and (osteopenia and osteoporosis) of the studied patients

		Osteopenia (n=52)	Osteoporosis (n=12)	P value
Vit K2 (nmol/L)	**Median**	0.9	0.055	**0.001***
	IQR	0.09 - 2	0.05 - 0.33	

*: Significant as P value≤0.05.

There was no correlation between vit K2 and (CTSK and P1NP) but there was a positive correlation between vit K2 and DEXA -T-score (r=0.271 and P value = 0.03). This positive correlation meaning that with increasing level of vit K2, DEXA T- Score is increased and degree of osteoporosis is decreased.

Table 4: Correlation between Vit K2 and (CTS-K, P1NP and DEXA -T-score) of the studied patients

	Vit K2 (ng/ml)	
	r	**P**
CTSK (pg/ml)	-0.187	0.140
P1NP (mcg/L)	0.166	0.191
DEXA -T-score	0.271	**0.030**

r: Correlation coefficient, CTSK: Cathepsin K, P1NP: Procollagen type 1 N-terminal propeptide.

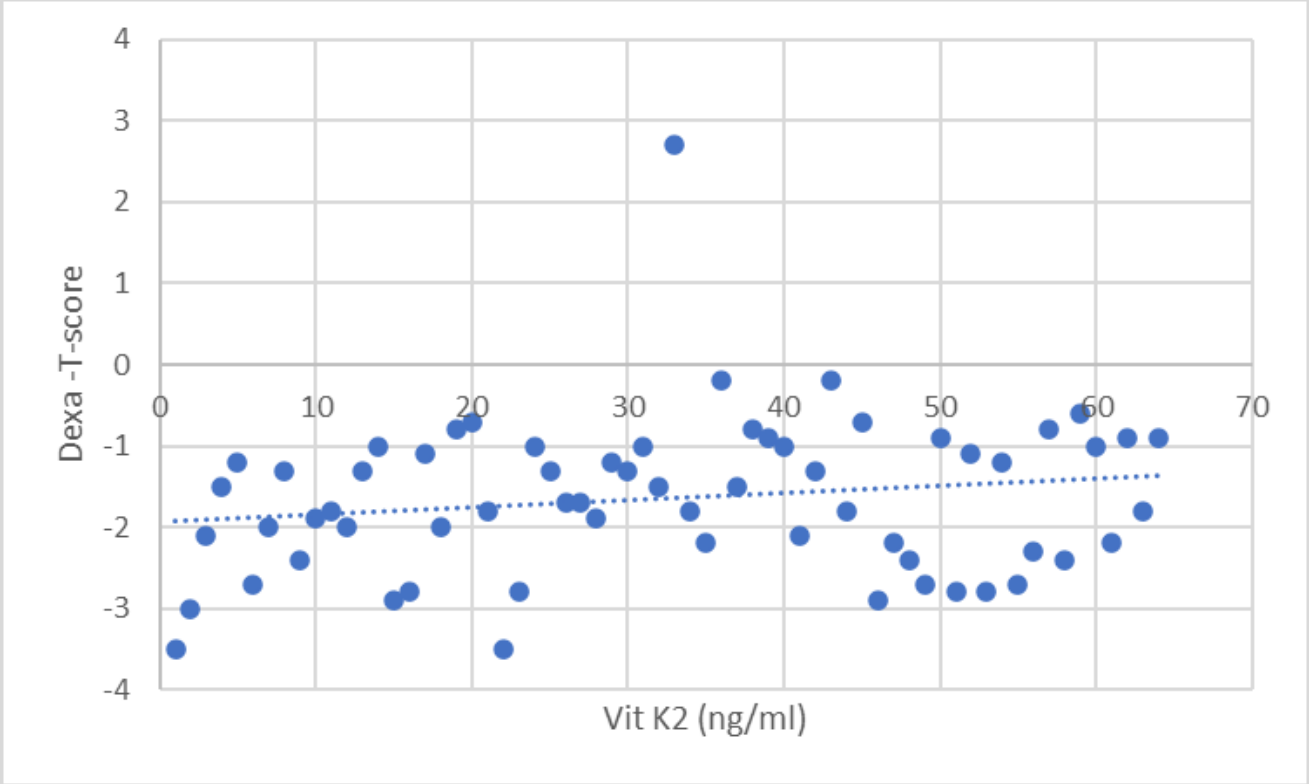

Figure 1: Correlation between Vit K2 and DEXA -T-score

There was no correlation between CTSK and P1NP level in HD patients

Table 5: Correlation between CTSK and (P1NP, DEXA -T-score) of the studied patients

	CTSK (pg/ml)	
	r	**P**
P1NP (mcg/L)	-0.108	0.398
DEXA -T-score	-0.492	**<0.001**

r: Correlation coefficient, CTSK: Cathepsin K, P1NP: Procollagen type 1 N-terminal propeptide.
There in significantly negative correlation between Cathepsin-K level and degree of osteoporosis (presented by DEXA T-Score) P **<0.001** meaning that with increasing level of Cathepsin -K, DEXA T- Score is decreased and degree of osteoporosis is increased.

Table 6: Correlation between P1NP and Dexa -T-score of the studied patients

	DEXA -T-score	
	r	**P**
P1NP (mcg/L)	0.2406	0.055

r: Correlation coefficient, CTSK: Cathepsin K, P1NP: Procollagen type 1 N-terminal propeptide.
No significant correlation between P1NP level and degree of osteoporosis (measured by DEXA- T-Score)

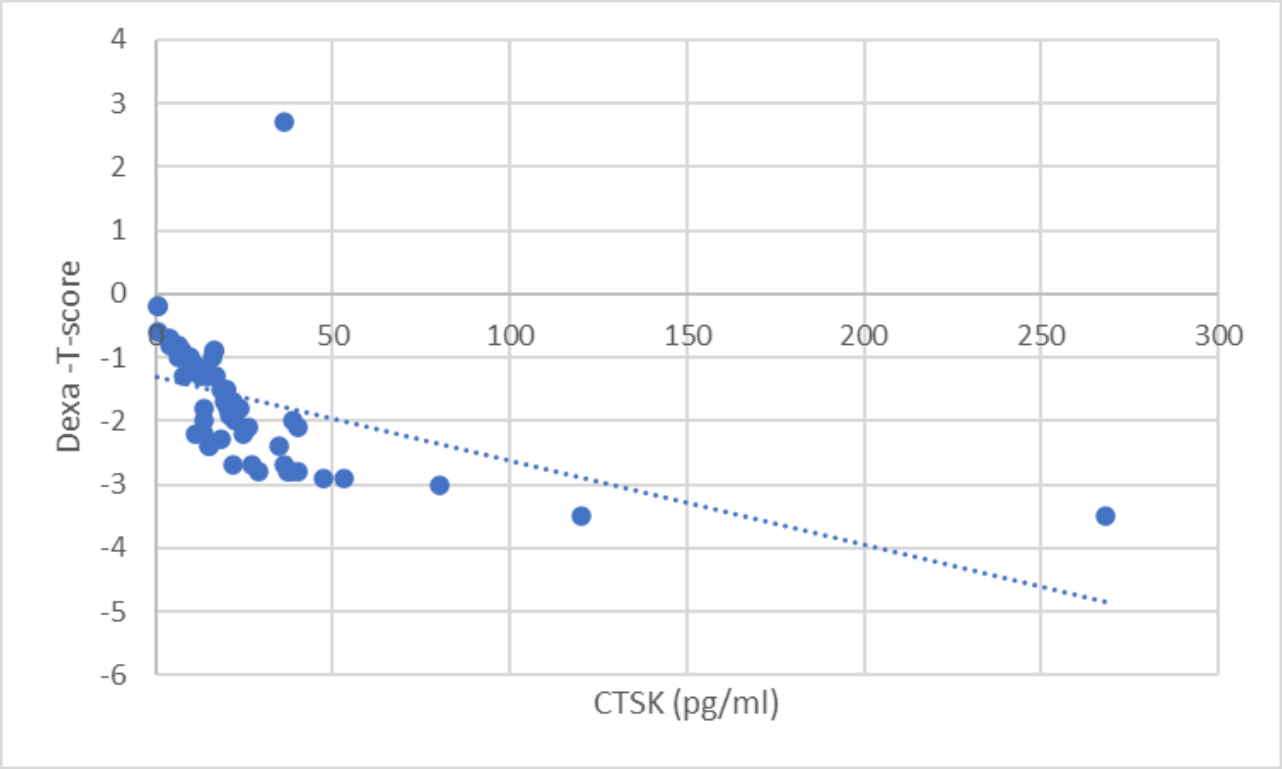

Figure 2: Correlation between CTS-K and DEXA -T-score

4. Conclusions

Vitamin K2 level in CKD patients on hemodialysis patients is important for bone health and has close relation to degree of osteoporosis, so giving Vit K2 supplementation is effective in protection and preventing of osteoporosis in hemodialysis patients. Treating risk factors increasing level of bone resorption enzymes as CTHK in hemodialysis patients is important for protection and preventing of osteoporosis in these patients. More high quality, longer-term trials are needed to prove benefits for preventing and treating osteoporosis, improving bone mineral density, and preventing fractures.

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Interest of conflict: None

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