Journal of Surgery and Trauma 2025; 13(3):103-110.
jsurgery.bums.ac.ir

ORIGINAL ARTICLE

Serum Vitamin D Levels and Their Association with Osteoporosis in Kidney Transplant Recipients: A Cross-Sectional Study in Birjand, Iran, in 2022

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Received: February 18, 2025 ***Revised: August 03, 2025*** ***Accepted: August 16, 2025***

Abstract

Introduction: Chronic Kidney Disease (CKD) is associated with mineral metabolism disorders, such as calcium, phosphorus, and calcium-regulating hormones. Moreover, the need to prescribe and use corticosteroid compounds increases the risk of osteoporosis in these patients. Therefore, this study aimed to investigate the serum level of Vitamin D and its relationship with osteoporosis in kidney transplant patients (KTPs) in Birjand, Iran.

Methods: This descriptive-analytical cross-sectional study included 50 KTPs in Birjand, Iran, who had passed at least one year after a kidney transplant. Vitamin D serum level and bone mineral density (BMD) were measured by the Dual-Energy X-Ray Absorptiometry method. Data analysis was done using SPSS software (version 19) and statistical tests, such as Fisher's exact test, chi-square, independent samples t-test, and Pearson correlation coefficient at α=0.05 level.

Results: Vitamin D level showed no significant relationship with BMD, T score, and Z score. The mean of Z SPINE, Z RAD, T SPINE, T HIP, and T RAD in women was significantly higher than that of men (P<0.05). Moreover, no statistically significant linear correlation of vitamin D levels was observed with T-score and Z-score indices according to age in the studied patients. Finally, the results showed no significant relationship of Vitamin D levels with BMD, T score, and Z score.

Conclusion: Considering that similar studies have been conducted at a limited level, it is suggested that more comprehensive studies be conducted to more accurately investigate and determine the relationship between Vitamin D and osteoporosis in KTPs.

Key words: Kidney transplantation, Osteoporosis, Vitamin D

Introduction

Chronic Kidney Disease (CKD) is known as one of the most important causes of death and suffering in the world, and due to the increase in underlying diseases, such as obesity and diabetes, the number of patients with CKD is also increasing (1). It is estimated that more than 434.3 million people in Asia are suffering from CKD (2), and in Iran, the prevalence of CKD in the general population is estimated at 15.14% (3). In general, a progressive and irreversible condition called CKD can cause end-stage renal failure (ESRD) that necessitates dialysis or kidney transplantation (4), and 20.4% of patients with stage 5 CKD after 2.5 years of starting treatment for ESRD receive a kidney transplant (5).

[DOI: 10.61882/jsurgtrauma.13.3.103]

Kidney transplantation in patients with ESRD offers a chance to improve kidney function, reduce the mortality rate in these patients, and improve the physical independence and quality of life of these patients (6).

CKD with metabolic bone disease can lead to osteoporosis and increased fracture risk, which may be more than double that of the general population (7). In addition, the consequences of fracture are more severe in CKD patients. Length of hospital stay and mortality in cases of hip fracture and ESRD are longer than those in patients with normal kidney function (8). Bone loss is increasingly common after kidney transplantation, as survival rates improve (9). Vitamin D serum level is inversely correlated with kidney function, as well as increased mortality in CKD patients (10). Therefore, Kidney Transplant Recipients (KTRs) often experience low Vitamin D levels, especially in the early stages of transplantation. As the glomerular filtration rate (GFR) declines, the active form of Vitamin D also decreases (7). On the other hand, CKD is a risk factor for Vitamin D deficiency and is especially common in KTRs and ESRD (11).

So far, the association between Vitamin D status and kidney complications, including osteoporosis, has not been well described in these patients, and the few studies conducted on the effectiveness of Vitamin D supplementation in KTPs have not reached definitive results (12). Therefore, to assess Vitamin D deficiency in KTPs with osteoporosis, further investigations are required. Accordingly, the present study was conducted to investigate the relationship between Vitamin D deficiency and osteoporosis in KTPs in Birjand, Iran.

Methods

The study population consisted of all KTPs in Birjand, Iran, who had undergone a procedure in 2022 and had at least one year of experience. Accordingly, those who had a file in the center for special diseases, those who had no history of osteoporosis prior to the transplant, and cases who were willing to participate in the survey were included in this cross-sectional descriptive-analytical study. On the other hand, patients with acute rejection (confirmed by biopsy within 3 months post-transplant), cases with resistant urinary tract infections to drug therapy, and those with increased serum creatinine levels were excluded from the study. In total, 50 patients eligible for the survey, as determined by the census, were included in the study.

After explaining the procedure and obtaining informed consent, venous blood samples were taken from the patients to determine the serum level of Vitamin D using Hologic 2022 technology and high-performance liquid chromatography (HPLC, Agilent 1100 series, America). The 25-hydroxyvitamin D ELISA was used to measure serum levels of 25-hydroxyvitamin D (25(OH)D). Bone density was measured using the Bone Mineral Density (BMD) test, Hologic bone density measuring device, series 2022 (Hologic Inc., Bedford, MA, USA). The results were interpreted by a rheumatologist for lumbar vertebrae and right femoral neck, and with the Dual-Energy X-Ray Absorptiometry (DXA) method by Hologic Horizon DXA (Hologic Inc., Bedford, MA, USA) scanner at the lumbar spine (L1-L4) and femoral neck. To ensure the accuracy and reliability of the measurements obtained by the DXA machine, lumbar spine phantoms were utilized for unit cross-calibration. The DXA method was used to measure bone density. In this method, X-rays are used to measure bone density. Some of the X-rays are absorbed by the bone (vertebral column or hip joint in the hip area), and some of them pass through the bone and exit the other side of the body. The higher the bone density, the more X-rays are absorbed and the fewer rays pass through, and the fewer rays reach the receiver. The radiation received by the receiver goes to a computer, where the radiation scale is converted into a bone density scale. Results were expressed as T-score (standard deviation [SD] relative to healthy young adults) and Z-score (SD relative to age-matched controls) based on World Health Organization criteria (13). The values were also expressed in percentiles by comparing both the reference peak and the age-matched groups. Bone density was classified as normal (T-score ≥ -1.0), osteopenia (T-score between -1.0 and -2.5 SD), moderate osteoporosis (T-score ≤ -2.5 SD), and severe osteoporosis (T-score ≤ -2.5 SD with at least one fracture site).

Based on plasma levels of Vitamin D (25-OH-D), values less than 30 ng/ml were considered Vitamin D deficiency (14). Data analysis was conducted using SPSS software (version 19). The normality of the data distribution was assessed with the Kolmogorov-Smirnov test. Based on these results, statistical methods, such as Fisher's exact test, chi-square test, independent samples t-test, and Pearson correlation coefficient, were used at a significance level of $\alpha = 0.05$.

The Birjand University of Medical Sciences Ethical Committee approved this study (IR.BUMS.REC.1401.184). In addition, participants were assured of the confidentiality of their information and the non-disclosure of their

[DOI: 10.61882/jsurgtrauma.13.3.103]

individual details.

Results

This study analyzed the data of 50 KTPs with a mean age of 43.8±13.7 years (age range: 14-83), and a mean BMI of 24.2±4.3 kg/m2 (range: 14.86-34.37). The mean duration of transplantation in the studied patients was 8.74±5.0 years (range: 3-22). Moreover, 52% of the studied patients were male, 38% were obese and overweight, 36% were 50 years old and older, and 34% had been 10 years or more since their transplant. Additionally, 50% of the studied patients had Vitamin D deficiency, and 34% had normal osteopenia status. More details are presented in Table 1.

Table 1. Distribution of demographic information, osteopenia status, and Vitamin D level in the studied patients

Variable		Frequency	%
Gender	Male	24	48
	Female	26	52
BMI (kg/m²)	Underweight	9	18
	Normal	22	44
	Overweight	13	26
	Obese	6	12
Age (year)	<50	32	64
	≥50	18	36
Year after transplantation	<10	33	66
	≥10	17	34
Osteopenia status	Normal	17	34
	Osteopenia	21	42
	Moderate osteoporosis	3	6
	Severe osteoporosis	9	18
Vitamin D level (ng/ml)	Normal	25	50
	Deficiency	25	50

The prevalence of osteopenia and osteoporosis in men was significantly higher than that of women (P=0.008), and in people ≥50 years, it was significantly higher than that in those <50 years (P=0.012). However, it did not show a significant difference in terms of Vitamin D level (P=0.55) and duration of transplantation (P=0.31). (Table 2).

In addition, there were no statistically significant differences regarding the level of Vitamin D according to age, gender, and duration of transplantation (Table 3).

Based on the results presented in Table 4, Vitamin D level showed no significant correlation with BMD, T score, and Z score.

The mean of Z SPINE (P=0.028), Z RAD (P=0.009), T SPINE (P=0.004), T HIP (P=0.022), and T RAD (P=0.004) in females is significantly higher than that of males, which showed a higher reduction of bone density in women and indicated that the bone is becoming weaker and more prone to fractures. In addition, the vitamin D levels in the studied patients showed no statistically significant relationship with BMD, T score, and Z score at different ages (Table 5).

Table 2. Comparison of the distribution of the frequency of osteopenia according to demographic variables

Variable		Osteopenia status				
		Normal (No (%))	Osteopenia (No (%))	Moderate Osteoporosis (No (%))	Severe Osteoporosis (No (%))	P-value*
Gender	Male	5 (19.2)	10 (38.5)	3 (11.5)	8 (30.8)	P=0.008
	Female	12 (50)	11 (45.8)	0 (0)	1 (4.2)	
Age (year)	<50	13 (40.6)	16 (50)	1 (3.1)	2 (6.3)	P=0.012
	≥50	4 (22.2)	5 (27.8)	2 (11.1)	7 (38.9)	
Vitamin D level (ng/ml)	Normal	7 (28)	13 (52)	1 (4)	4 (16)	P=0.55
	Deficiency	10 (40)	8 (32)	2 (8)	5 (20)	
Year after transplantation	<10	14 (42.4)	12 (36.4)	2 (6.1)	5 (15.2)	P=0.31
	≥10	3 (17.6)	9 (52.9)	1 (5.9)	4 (23.5)	

* Chi-square test

[DOI: 10.61882/jsurgtrauma.13.3.103]

Table 3. Comparison of the distribution of the frequency of Vitamin D level according to demographic variables

Variable		Vitamin D level		P-value*
		Normal (No (%))	Deficiency (No (%))	
Gender	Male	15 (57.5)	11 (12.3)	P=0.39
	Female	11 (41.7)	14 (58.3)	
Age (year)	<50	17 (53.1)	15 (46.9)	P=0.56
	≥50	8 (44.4)	10 (55.6)	
Year after transplantation	<10	17 (51.5)	16 (48.5)	P=0.76
	≥10	8 (47.1)	9 (52.9)	

* Chi-square test.

Table 4. Correlation of Vitamin D level with BMD, T score, and Z score in the studied patients

	BMD (g/cm^2)			T SCORE			Z SCORE		
	RAD	HIP	SPINE	RAD	HIP	SPINE	RAD	HIP	SPINE
r(Pearson correlation coefficient)	0.09	0.14	0.01	0.06	0.13	0.12	0.11	0.23	0.13
p-value	0.51	0.32	0.95	0.66	0.35	0.41	0.44	0.1	0.43

Table 5. Comparison of Vitamin D level and BMD, T score, and Z score indices according to gender and age in the studied patients

Variable		Vitamin D (ng/ml)	BMD (g/cm^2)			T SCORE			Z SCORE		
			RAD	HIP	SPINE	RAD	HIP	SPINE	RAD	HIP	SPINE
Gender	Male	32.7±12.5	0.59±0.11	0.64±0.14	0.87±0.21	-2.76±1.7	-1.78±1.1	-2.1±1.4	-2.42±1.6	-1.23±0.96	-1.69±1.57
	Female	29.5±8.9	0.55±0.14	0.73±0.2	1±0.32	-1.53±1.16	-1.1±1.1	-1.03±1.1	-1.33±1.19	-0.71±1.19	-0.81±1.13
	P-value*	0.3	0.28	0.08	0.08	0.004	0.022	0.004	0.009	0.09	0.028
Age (year)	<50	30.1±9.5	0.57±0.13	0.68±0.14	0.95±0.27	-1.98±1.4	-1.29±1.1	-1.4±1.3	-1.73±1.4	-1.03±1.12	-1.9±0.13
	≥50	32.5±13.3	0.59±0.12	0.69±0.24	-2.5±1.8	-2.5±1.8	-1.68±1.2	-1.94±1.4	-2.2±1.7	-0.9±1.1	-1.22±1.6
	p-value*	0.39	0.59	0.97	0.58	0.27	0.25	0.19	0.29	0.71	0.87

* Independent samples T-test

Discussion

This study investigated the serum level of Vitamin D and its relationship with osteoporosis in KTPs in Birjand, Iran, in 2022. No significant correlation was found between Vitamin D levels and BMD, contrasting with general population studies. Moreover, there was no statistically significant difference in the level of Vitamin D according to age, gender, and duration of transplantation. The prevalence of osteopenia and osteoporosis in males was significantly higher than that in females. Additionally, in individuals ≥50 years old, it was significantly higher than in those ≤50 years old; however, no significant difference was observed in terms of Vitamin D level or duration of transplantation. In the same vein, Vitamin D levels showed no significant correlation with BMD, T SCORE, and Z SCORE. However, the means of Z SPINE, Z RAD, T SPINE, T HIP, and T RAD in women were significantly higher than those of men (P<0.05). There was also no statistically significant correlation of Vitamin D levels with T SCORE and Z SCORE indices according to age in the studied patients.

The results of this study are consistent with the findings of studies by Restrepo Valencia et al. (2018) (15) and Battaglia et al. (2022) (16). In the mentioned studies, there was no significant relationship between Vitamin D deficiency and the degree of osteoporosis. In the present study, comparisons of osteoporosis were made based on the four classifications of osteoporosis (normal, osteopenia, osteoporosis, and severe osteoporosis). On the other hand, the results of this study are not consistent with the results of the survey by Gheiasi et al. (2022) (17) since in that study, a significant relationship between osteoporosis of the femoral neck and Vitamin D deficiency was reported in patients aged <50 years. They concluded that patients with Vitamin D levels of 15-30 ng/mL are 90% less likely to develop femoral osteoporosis, compared to those with Vitamin D levels <15 ng/mL. They also stated that this finding might be attributed to Vitamin D supplementation in patients younger than 50 years. Previously, the association between low serum Vitamin D levels and decreased BMD and increased bone loss was reported in the general population (18). This is despite the fact that in patients over 50 years, factors, such as advancing age and duration of prednisolone use, were known to be risk factors for increased risk of osteoporosis and osteopenia at the femoral neck, and the duration of prednisolone use was associated with

[DOI: 10.61882/jsurgtrauma.13.3.103]

osteoporosis at the lumbar spine (17). Unlike studies in high-latitude countries, our cohort in sunny Iran may have different Vitamin D metabolism.

Although a study similar to the present study has not been conducted in Iran, the results of this study differ from those of Mahdavi et al. (19) regarding the relationship between age and gender. However, in terms of the lack of relationship between the time of the transplant, they are aligned with each other. Additionally, the results of the study by Sharifipour et al. (20) are in line with the findings of the current study, regarding the lack of correlation between the duration of transplantation and the occurrence of bone mineral disorders after transplantation. In the present study, our findings differ from those of Mojahedi et al. (21) and Davachi et al. (22) regarding the transplant timing. KTRs face significant bone strength impairments, with 32% having osteopenia and 15% having osteoporosis (23). Post-transplant, the skeleton undergoes changes that increase the risk of fractures, especially in the early period. Hip fracture rates among transplant recipients are three times higher than those among dialysis patients during the first three years after transplantation (24).

The most important reason mentioned for the exacerbation of osteoporosis in KTPs is the use of high-dose glucocorticoids, which, unfortunately, could not be investigated in this study due to the same drug dosage in the studied subjects. However, recent bone biopsy studies suggest that lower-dose glucocorticoid-based immunosuppressive regimens are associated with less severe disturbances in bone quality, compared to previous studies in patients managed with higher-dose glucocorticoid-based regimens. This may be explained by the shift in immunosuppressive regimens over the past few decades, favoring reduced glucocorticoid doses or complete discontinuation of glucocorticoids (25). Glucocorticoids are toxic to the skeleton, and exposure to glucocorticoids is a significant risk factor for fracture. Thus, the epidemiology of bone disease and fracture after renal transplantation has changed in parallel with the decreased use of glucocorticoid-based immunosuppression (25). Several prospective studies in recipients managed with low-dose or early-discontinuation regimens of glucocorticoids have reported stable or increased BMD in the central skeleton (i.e., spine and pelvis) during the first 12 months of transplantation (26). In contrast, BMD decreased in the peripheral skeleton (i.e., radius and tibia) (26).

Along with glucocorticoids, Vitamin D deficiency is also considered a factor influencing the occurrence or exacerbation of osteoporosis in these patients, due to its high prevalence in KTPs and its essential role in the development of osteoporosis in the general population. Therefore, prescription of Vitamin D supplements is part of the treatment management plan in this group of patients. However, to date, few studies (20, 27) have investigated the causal relationship between Vitamin D deficiency and osteoporosis in KTPs. Consistent with the present study, no causal relationship was found, and Vitamin D supplementation has not demonstrated a significant effect on osteoporosis.

Although the small sample size in all these studies is mentioned as a primary limitation, it appears that the process of osteoporosis in these patients is complex, and additional risk factors should be investigated. In addition, some studies have considered the possible role of immunosuppression in Vitamin D metabolism (28).

Generally, Vitamin D deficiency is a highly prevalent issue following transplantation, affecting as many as 80% of recipients within the first three months and often persisting long-term (29). In laboratory studies, Vitamin D has been shown to play a crucial immunoregulatory role. It modulates immune function by reducing the maturation and antigen-presenting capacity of dendritic cells, while also promoting the differentiation of regulatory T cells. These actions help to improve pathogen clearance and support the proliferation of immunosuppressive cells (30). Consequently, it is hypothesized that a Vitamin D deficiency in immunosuppressed individuals could lead to decreased transplant tolerance, an increased risk of infections, and a higher chance of malignancies. In addition to its immune functions, a lack of Vitamin D is also a known cause of hypocalcemia and subsequent bone loss (30).

Normal levels of 25-hydroxyvitamin D (30 to 40 ng/ml), or calcidiol, are crucial for KTPs to produce the active form of Vitamin D (31). Low Vitamin D levels in KTPs can lead to several complications, the most significant being: a) delayed graft function and a higher risk of acute rejection, which can lead to accelerated loss of kidney function, tubular atrophy, interstitial fibrosis, and a low GFR one year after transplantation (32); b) stronger tendency to develop opportunistic viral infections, such as polyomavirus (33) and bacterial infections, including urinary tract infections (34); c) significant symptoms of depression and fatigue (35); d) persistence of secondary hyperparathyroidism (36); e) diabetes mellitus (37); and f) the risk of cancer (38).

The primary causes of sustained Vitamin D deficiency induced by transplantation are decreased allograft function and higher FGF-23 levels (29). Low

[DOI: 10.61882/jsurgtrauma.13.3.103]

Vitamin D levels in KTPs can be caused by low exposure to sunlight, low consumption of Vitamin D-rich foods, and the type of immunosuppressive treatment prescribed. Patients who receive cytostatic therapy (e.g., azathioprine and mycophenolate) are advised to limit their sun exposure in order to avoid skin neoplasms (39). Studies investigating serum levels of native Vitamin D in various groups of kidney transplant recipients have shown a higher prevalence of Vitamin D deficiency, reported to be as high as 97%, in regions of the Northern Hemisphere, where sunlight exposure is limited (40).

Therefore, extrapolating the results related to Vitamin D deficiency in these regions to tropical regions should be done with caution. In Iran, the study of seasonal changes in Vitamin D in KTPs revealed no seasonal variations. However, during the summer, patients avoided excessive sun exposure and used sunscreen (40). In general, the lack of correlation between Vitamin D deficiency and osteoporosis in KTPs requires further investigation and consideration of the mediating role of other factors.

When interpreting the results, several limitations of this study should be considered: a) A cause-and-effect relationship cannot be established due to the cross-sectional design of the study and the lack of data on cumulative glucocorticoid doses, pre-transplant BMD, and parathyroid hormone levels; b) Although adjustments were made for several known confounding variables, unmeasured factors may still have influenced the results, including cumulative glucocorticoid doses, pre-transplant BMD, parathyroid hormone levels, individual dietary habits (particularly calcium intake), as well as precise levels of physical activity and sun exposure; c) As this was a single-center study, the findings may not be fully generalizable to all KTPs; d) The study did not assess longitudinal changes in Vitamin D levels or bone density following transplantation; e) The relatively small sample size limits the robustness of the conclusions.

Conclusions

The study revealed no significant relationship between Vitamin D deficiency and osteoporosis in KTPs. However, due to the high prevalence of metabolic disorders and especially the decrease in bone density, as well as the need to prescribe and consume corticosteroid compounds, KTPs are at high risk of osteoporosis. Conducting more studies with a larger sample size and considering other factors is necessary for the early detection of osteoporosis and identification of the effect of Vitamin D deficiency on bone quality in these patients. Additionally, a prolonged study would be necessary to monitor these changes and their impact on long-term outcomes.

Acknowledgments

None.

Funding

This study did not receive any financial support.

Conflict of Interest

The authors state no conflict of interest.

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[DOI: 10.61882/jsurgtrauma.13.3.103]