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Serum phosphorus levels and fracture following renal transplantation.

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Mini abstract:

This study examined the risk factors for fracture post-renal transplantation. We observed a significant association between declining femoral neck areal bone mineral density and serum phosphorus levels with fracture. This previously unreported observation requires further evaluation in prospective studies.

Abbreviations

RTRs: Renal transplant recipients

DXA: Dual energy x-ray densitometry

ESKD: End stage kidney disease

BMD: Bone mineral density

FN: Femoral neck

LS: Lumbar spine

TB: Total body

aBMD: areal bone mineral density

FGF-23: Fibroblast growth factor-23

PTH: Parathyroid hormone

SPK: Simultaneous pancreas and kidney

TSH: Thyroid stimulating hormone

eGFR: Estimated glomerular filtration rate

TmP/GFR: Tubular reabsorption of phosphate normalised for glomerular filtration rate

Abstract

Purpose: Increased fracture rates are observed in renal transplant recipients (RTRs) compared with the general population. Risk factors include age, diabetes, dialysis vintage, immunosuppression, and mineral and bone disorders (1). Low serum phosphorus levels occur post-transplantation; however its relationship with fracture risk has not been evaluated. The purpose of this study was to evaluate risk factors for fracture in RTRs at a single tertiary referral centre.

Methods: A retrospective cross-sectional analysis of 146 patients (75M, 71F) who had been referred for dual energy x-ray densitometry (DXA) post-renal transplantation was performed. Aetiology of end stage kidney disease (ESKD), duration of dialysis, parathyroidectomy history, immunosuppression regimen, bone mineral density (BMD), biochemistry and fractures were documented. Statistical analyses included univariable and multivariable regression.

Results: The mean age of patients was 54 years and mean time post-transplantation 6.7 years. 79 fractures occurred in 52 patients (35%), with 40 fractures occurring post-transplantation. Ankle/foot fractures were most common (48%). Lower serum phosphorus levels and declining FN T-score and were associated with fractures in both univariable and multivariable regression analyses after adjusting for age, gender, weight, eGFR and pre-transplant history of fracture ($p=0.011$ and $p=0.042$ respectively). The relationship between serum phosphorus and fracture remained significant independent of FN T-score, parathyroid hormone levels, parathyroidectomy status and prednisolone use.

Conclusion: Fracture was common post-renal transplantation. Lower serum phosphorus levels and declining FN T-scores were associated with fractures. The mechanism of this previously unreported observation requires further evaluation in prospective studies.

Introduction

Kidney transplantation is the gold standard treatment for patients with end stage kidney disease (ESKD), with ensuing improvements in quality of life, reduced morbidity and mortality and reduced financial burden compared with dialysis (2, 3). Although renal transplantation can reverse many of the mineral and bone disturbances seen in ESKD, fractures post-transplant remain a significant complication (4). Patients with ESKD have an increased risk of fracture compared with the general population; and hip fracture rates post-renal transplantation exceed those on dialysis (4-6). Advancing age, pre-existing and persisting mineral and bone disorders as a consequence of ESKD, glucocorticoid immunosuppression, degree of graft function and underlying diabetes mellitus are recognized risk factors for fractures post-renal transplantation (1).

Low bone mineral density (BMD) as measured by DXA is an established risk factor for fractures in the general population (7). BMD declines rapidly in the first 6-12 months following renal transplantation and has been associated with increased fractures (8). Studies of long-term changes in BMD post-transplantation show conflicting results, with studies demonstrating continued, stabilisation or incremental changes in BMD (9, 10). DXA calculates a two dimensional areal BMD of a three dimensional structure, and therefore cannot provide information with respect to bone microarchitecture and bone quality that also contribute to bone strength and fractures (11). Glucocorticoid immunosuppression, persisting mineral and bone disorders such as hyperparathyroidism and vitamin D deficiency may all compromise bone quality and fragility, and may not be appreciated on DXA imaging (10, 12). The validity of DXA imaging to predict fracture

1 risk in the renal transplant population is not well established. Some studies have shown no significant
2 association between BMD and vertebral fractures (10) but others have demonstrated its prognostic
3 value for fracture prediction (13, 14).

4
5 Although many mineral and bone disorders can persist or arise *de novo* post-transplantation, the role
6 of phosphorus in post-transplant fractures has not been well evaluated. Hypophosphataemia is
7 common in the early post-transplant period. Renal phosphate wasting occurs due to high levels of the
8 two phosphaturic hormones, fibroblast growth factor-23 (FGF-23) and parathyroid hormone (PTH)
9 (15). Elevations in these hormones occur with declining renal function in an attempt to counter
10 phosphate retention and maintain serum phosphorus homeostasis (16). At 1 year post-transplantation,
11 most patients with good graft function normalise their serum phosphorus levels, with concomitant
12 reduction in FGF-23 and PTH levels (15). Despite normal serum phosphorus levels, persistent renal
13 tubular phosphorus leak has been reported in patients for up to 10 years post-transplantation (17, 18).
14 However, implications of low phosphorus levels on BMD and fractures in the short and longer term
15 have not been specifically examined.

16
17 We therefore performed a cross-sectional study of adult renal transplant recipients to investigate the
18 impact of bone-related biochemistry, clinical and DXA parameters on fractures.

20 **Methods**

21 *Patients*

22 We reviewed the medical records of all patients from the renal transplant registry at Monash Health.
23 Patients with a renal transplant or a simultaneous pancreas and kidney (SPK) transplant with a
24 minimum of one post-transplantation DXA between 2012-2016 were included. Information regarding
25 aetiology of ESKD, pre-existing diabetes mellitus, previous parathyroidectomy, osteoporosis
26 treatment with anti-resorptive agents (oral or intravenous bisphosphonates and denosumab) or
27 teriparatide, and hypogonadism were obtained from medical records. Male patients with
28 hypogonadism who were on androgen replacement and had serum testosterone levels within the
29 normal range were included in the analyses. The Monash Health Human Research Ethics Committee
30 approved the study.

32 *Time on dialysis*

33 Time on dialysis (months) was accurately obtained using the Australia and New Zealand Dialysis and
34 Transplant Registry (ANZDATA).

36 *Biochemical parameters*

Measurements of serum calcium, phosphorus, creatinine, albumin, haemoglobin, thyroid stimulating hormone (TSH) and PTH were performed on the Beckman Coulter UniCel (Beckman Coulter, Brea, CA, USA) and the Diasorin Liaison (DiaSorin Inc., Stillwater, MN, USA) was used to measure serum 25-hydroxyvitamin D (25(OH)). Estimated GFR (eGFR) was based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (19). Biochemical parameters were documented during a routine clinical visit within six months of DXA imaging.

BMD measurement

BMD was measured at the lumbar spine (L1-L4), femoral neck (FN) and total body (TB). BMD was expressed as grams per centimeter squared (g/cm^2); T-score (standard deviation relative to young healthy adults) and Z-scores were calculated relative to gender- and age-specific norms. All measurements were obtained using a GE Lunar Prodigy (Madison, Wisconsin, software version 12) at a single centre between 2012-2016. Anthropometric measures of age, weight, and height were also documented at the time of DXA scanning. BMI (kg/m^2) was calculated by dividing weight (kg) by height squared (m^2).

Fractures

Fractures were confirmed on medical records or radiological reports. Vertebral fractures were a composite of clinical and radiological fractures, and all were validated on spinal x-ray by a radiologist with evidence $>20\%$ vertebral height loss. Non-vertebral fractures were verified through a combination of documentation in the medical records or X-ray reports. Analyses was performed according to fracture history post-transplantation.

Statistical analysis

We expressed continuous data as mean $\pm$ standard deviation and categorical data as percentages. Categorical data were compared using the chi-square test. Continuous data were evaluated for normality before statistical testing. We performed univariable logistic regression analysis followed by multivariable analyses to adjust for relevant potential confounders. A p-value <0.05 was considered statistically significant and all tests were two-sided. Analyses were conducted using IBM SPSS 20 (version 20.0, Armonk, NY).

Results

Patient Characteristics

75 (51%) males and 71 (49%) females, with 45 (31%) being post-menopausal, had at least one BMD measurement post-renal transplantation (Table 1). Mean age was 54.8 years in males, 41.4 years in premenopausal females and 59.6 years in the postmenopausal females. The most common cause of ESKD was glomerulonephritis 38%, followed by diabetes mellitus in 27%. Sixteen percent had a SPK transplant and a total of 36% had pre-existing diabetes mellitus. The median time on dialysis was 38 months, and 27 patients received pre-emptive living donor renal transplant. Eighty-six percent of patients were on long-term maintenance prednisolone and 95% were receiving a calcineurin inhibitor. The most common maintenance immunosuppression regimen consisted of a combination of prednisolone, calcineurin inhibitor (tacrolimus or cyclosporine), and antimetabolite (azathioprine or mycophenolate). Twenty-three patients were on dual-agent immunosuppression and 8 patients were receiving a mammalian target of rapamycin (mTOR) inhibitor (everolimus or sirolimus). 21 patients (14%) were taking specific anti-osteoporotic medications, 18 bisphosphonates, 2 were on subcutaneous denosumab injections and one patient was on an oral combination of bisphosphonate and subcutaneous teriparatide injections. 11 patients had a history of prior parathyroidectomy and no patient was receiving cinacalcet post-transplantation.

Fractures

There were 79 documented fractures amongst 52 patients (35%) with 40 of these patients sustaining fractures post-transplantation. 12 patients had a history of fracture pre-transplantation. The mean time of fracture post-transplant was 4 years with 75% occurring within the first 5 years after transplantation. The majority of fractures were lower limb fractures, with ankle/foot fractures accounting for 46%. 5 vertebral fractures were documented and the other fracture sites included hand/wrist (14), shoulder (5), rib (9), patella (3), hip (3) and sternum (2) (Table 2).

Biochemical parameters

Taken at the time of DXA imaging, the mean ($\pm$ SD) eGFR was 54.94 ± 21.98 mls/min/1.73², adjusted calcium 2.40 ± 0.15 mmol/L, phosphorus 1.10 ± 0.33 mmol/L and ALP 88.33 ± 62.42 U/L. The median and 25-75 interquartile range for PTH and 25(OH)D were 8.5 (5.3-12.9) and 60.5 (43.2-75.9) respectively (Table 1).

BMD parameters

BMD was performed at a median time of 5.1 (IQR 2.3- 9.3) years post-transplantation, as part of patients' routine post-transplant care. Few patients (<5%) were referred for DXA imaging with a secondary indication including hyperparathyroidism, post-menopausal state or history of hypogonadism or early menopause. In post-menopausal women and all men, T-scores were < -1.0 at

the FN, LS and TB in 75%, 37% and 49% of patients respectively. In premenopausal women, 19%, 15% and 8% had Z-scores ≤ -2 at the FN, LS and TB respectively, indicating low bone mass. In postmenopausal women, T-scores were < -1.0 in 84% at the femoral neck, and 55% at the lumbar spine and total body (Table 3). The use of prednisolone was significantly associated with lower LS aBMD in a linear multivariable regression analysis adjusting for age, gender and weight ($p=0.035$, $\beta -0.164$) (Table 4) but not with FN or TB aBMD ($p=0.112$ and $p=0.165$ respectively). Pre-existing diabetes, time on dialysis and parathyroidectomy status was not associated with any BMD parameter.

The clinical, biochemical and BMD characteristics of patients according to the presence of fracture post-transplantation are presented in Table 1. Compared with patients without fracture post-transplant, pre-existing diabetes mellitus was more common in patients with fracture. There was also a statistically significant difference in serum phosphorus levels, creatinine, adjusted calcium, FN T-score and FN areal BMD between fracture and non-fracture patients. Analysis of SPK patients compared to renal-transplant-only recipients, demonstrated these patients to be younger (mean 55.1 versus 47.9 years, $p=0.001$), have a higher incidence of fracture (48% versus 24%, $p=0.017$), and a shorter duration of dialysis pre-transplantation (median 39.4 versus 17.3 months, $p=0.006$). Their eGFR was higher (70.7 versus 52 mls/min; $p<0.001$) and they also had lower femoral neck and total body BMD values ($p=0.011$ and $p=0.006$).

In multivariable logistic regression analyses, after adjusting for age, gender, weight, pre-transplant fracture history and eGFR, FN areal BMD and FN T-score remained significantly associated with post-transplant fractures (OR 1.57 (95% CI 1.01- 2.42), $p=0.045$ and OR 1.62 (95% CI 1.02- 2.59), $p=0.042$ respectively). Univariable analysis showed that lower serum phosphorus and creatinine, and higher adjusted calcium to be significantly associated with fracture post-transplantation ($p=0.002$, $p=0.022$ and $p=0.013$, respectively) (Table 5). However, phosphorus was the only significant predictor of fracture after adjustment in the multivariable model (OR 2.64 (95% CI 1.24-5.75), $p=0.011$) (Table 5). The relationship of phosphorus and fracture was not altered by adjusting for PTH, 25(OH)D or ALP levels, parathyroidectomy status, prednisolone or tacrolimus use nor was it associated with dialysis vintage. When serum phosphorus and FN T-score were both included within the multivariable model, phosphorus was the strongest predictor for fracture (OR 2.62 (95% CI 1.21-5.75), $p=0.015$) (Table 6). The presence of diabetes mellitus was not associated with serum phosphorus levels ($p=0.82$) and when the presence of diabetes included in the multivariable model, phosphorus remained a significant risk factor for fracture ($p=0.005$).

Discussion

We performed a cross-sectional analysis of renal transplant recipients to evaluate clinical, biochemical and DXA parameters as risk factors for fracture. We demonstrated patients with fractures had lower serum phosphorus levels, FN aBMD and FN T-score. We found that for every standard deviation reduction in serum phosphorus, there was a 2.6 fold increase in fracture risk. Additionally, fracture risk was increased by 1.6 fold for each standard deviation lower FN aBMD or FN T-score.

27% of patients experienced a fracture post-transplantation with ankle/foot fractures being most common. Although there was a greater number of foot fractures in males, when foot and ankle fractures were combined as 'lower extremity fractures', there was no longer a gender difference.

It is important to note that our patient cohort had been referred for DXA imaging, which may overestimate fracture prevalence due to referral bias. Despite this, these findings are consistent with existing studies that have reported elevated rates of peripheral fractures in patients following renal transplantation (14, 20). Fracture prevalence of 22.5% was reported in a five year follow-up study (20) and up to 52% in patients with SPK transplant (21). In these studies, patients with peripheral fractures frequently had coexisting vertebral fractures (14), and vertebral fractures rates have been reported in up to 60% of long-term RTRs (8). In our cohort, 3 of 5 patients that had vertebral fractures also had peripheral fractures (ribs, ulna and sternum). We did not have routine thoraco-lumbar radiographs to screen for asymptomatic vertebral fractures, and thus vertebral fractures are likely to have been under-reported.

Lower limb fractures represent skeletal sites predominately composed of cortical bone that are sensitive to changes in PTH levels. It has been reported that persistent hyperparathyroidism post-transplant may contribute to reduced cortical BMD, and the elevated fracture risk in this population group (22). Although our patient cohort had a median PTH level approximately 1.5 times above the upper limit of normal, we found no association between PTH levels and fractures, or PTH with any DXA parameter. Furthermore, the lower incidence of typical osteoporotic fractures (hip/ humerus) in our cohort may be due to their younger age compared to the general osteoporotic population.

Amongst demographic parameters, older age and pre-existing diabetes mellitus were associated with fractures. Advancing age is a well-recognized risk factor for fracture in the general population, in patients with ESKD and post-renal transplantation (1). There was no significant difference in the age or BMD measurements between patients with and without pre-existing diabetes mellitus. There was also no difference in these parameters between diabetic patients with and without fracture. Diabetes mellitus has been shown in many large epidemiological studies to be a risk factor for fracture with or without renal transplantation (1, 4, 5). Studies in the general population with diabetes mellitus, have demonstrated adverse effects on bone quality predisposing to fragility (23). The association of SPK

transplantation with fracture may reflect their underlying diabetes mellitus, a known risk factor for fracture in RTRs. No other demographic parameter including time of fracture post-transplantation, dialysis vintage, parathyroidectomy status, prednisolone and anti-osteoporotic medication use were significantly associated with fractures.

Although creatinine was associated with fractures in univariable analysis, it was not significant after adjusting for age, gender and weight. Higher adjusted calcium levels were associated with increased fractures ($p=0.013$) on univariable analysis, despite the adjusted calcium levels in our cohort being within the normal range. However, this relationship was no longer significant in the multivariable analysis. This contrasts to the association of phosphorus levels with fractures that remained significant following adjustments in the multivariable analysis. Although elevated calcium and low phosphorus levels would normally suggest the presence of high PTH levels, this association was not present in our study. Repeat analyses was performed with a second serum phosphorus level, obtained within a mean of 70 days of the first serum phosphorus level, and the association between lower serum phosphate and fracture remained significant. Univariable analysis using the mean phosphorus level, showed lower phosphate level was significantly associated with fracture ($p=0.007$), and phosphorus remained a significant risk factor for fracture using the same adjusted multivariate analysis ($p=0.015$) (data not shown).

To our knowledge, the association of lower serum phosphorus levels with increased fracture risk in RTRs has not previously been reported. Hypophosphataemia is common early post-transplantation occurring in up to 90% of patients (24). The predominant mechanism is renal phosphorus loss mediated by persistence of high levels of FGF-23 and PTH (24), although low calcitriol levels may also contribute (25). FGF-23 declines rapidly in the first three months post-transplantation reaching similar values to those in chronic kidney disease (CKD) patients with equivalent renal function by 12 months. PTH levels also decline, but often remain higher than CKD equivalent patients (15). Phosphorous levels decline in parallel with FGF-23 and usually normalise by 12 months post-transplantation, but a small percentage may continue to have hypophosphataemia (15, 17). Despite normal serum phosphorus levels, Tomida *et al.* showed RTRs have ongoing excessive renal phosphate loss through reduced tubular reabsorption of phosphate normalised for glomerular filtration rate (TmP/GFR) compared to pre-dialysis CKD equivalents for up to 10 years following their transplant (18). The chronic urinary phosphorus leak has been associated with higher PTH levels, longer dialysis vintage, and although it has not been associated with FGF-23 levels (18), this remains a conceivable mechanism. In our study, there was no relationship between fractures and PTH levels, and there was no association between serum phosphorus levels, PTH or dialysis vintage. Calcineurin inhibitors have also been implicated as a cause of renal phosphorus leak. This has been supported by the finding that cyclosporin reduces expression of the type II sodium/phosphorus (Na/Pi) cotransporter in renal

tubules of mice with subsequent phosphorus wasting (26). This observation is yet to be validated in humans. We found no association between phosphorus levels and CNI use, or CNI use with fractures, however there was insufficient power for any conclusions to be drawn given only 9 participants (6%) were not taking a CNI.

Persistent renal phosphorus leak following transplantation presents a feasible explanation for skeletal fragility in this cohort. Skeletal mobilization of phosphorus stores occurs in order to maintain normal serum phosphorus homeostasis, with consequent effects on bone. Alternatively, it may be that low serum phosphorus is a 'marker' of high FGF-23 levels, and FGF-23 is the responsible factor for abnormal bone metabolism and increased fragility. A state of 'tertiary hyperphosphatonism' has been hypothesized, whereby longstanding elevations in FGF-23 pre-transplant may result in autonomously high levels post-transplantation (27). Others have postulated that FGF-23 may have phosphorus independent mechanisms on bone metabolism (28).

In other bone disorders with abnormal phosphorus metabolism such as hypophosphatemic rickets and drug-induced hypophosphatemia, DXA imaging shows low BMD due to reduced bone mineralization (29, 30). Extrapolating from these disorders, it would be anticipated that low phosphorus levels in RTRs would also be associated with reduced BMD as measured by DXA. In our study, serum phosphorus levels did not correlate with any BMD parameter. When phosphorus and FN T-score were both included in the multivariable analysis, phosphorus was the strongest predictor for fracture and FN T-score was no longer significant. The relationship between these variables remains unclear, but could reflect previous reports that BMD is a poor surrogate for the underlying histology and fracture risk in this population. No studies have demonstrated an association between lower serum phosphorus and or lower TmP/GFR with reflected changes to DXA parameters in RTRs—further studies are required to delineate if there is a relationship between serum phosphorus, TmP/GFR and BMD changes.

Phosphorus replacement is an attractive potential therapeutic avenue to reduce hypophosphataemia post-renal transplantation. However, the optimal phosphorus level in the acute and chronic setting after transplantation is unknown. There are significant concerns regarding calcification propensity and cardiovascular disease in these patients and studies have associated higher phosphorus with increased vascular calcification and cardiovascular mortality (31). Case reports have also suggested phosphorus replacement increases calcification in the transplanted kidney with adverse effects on graft function (32). Furthermore, phosphate replacement may be ineffective given the propensity to further stimulate FGF-23 secretion from the additional phosphate load. Despite these concerns, treatment of severe hypophosphataemia post-transplantation may be required to alleviate profound symptoms such as muscle weakness. There is currently insufficient evidence to guide phosphorus replacement after

transplantation, and prospective studies are required to establish optimal phosphorus levels and the safety and therapeutic benefit of replacement on cardiovascular and skeletal outcomes.

Limitations

We are mindful of the limitations of this study. This was a retrospective study and therefore causation cannot be demonstrated. As only patients with post-transplant DXA scans were included in the analyses this is likely to have introduced a selection bias. Conversely, the true prevalence of fractures, particularly vertebral fractures, may have been underestimated as routine spinal x-rays were not performed. Despite this and small number of total fractures, our study had similar fracture prevalence post-transplantation compared to prior studies (20). Additional factors that may contribute to fragility fractures such as smoking, alcohol consumption, physical exercise, familial history of fractures and other risk factors for falls were also not available.

We were unable to extract the cumulative dose exposure to prednisolone due to limitations of a cross-sectional nature of the study. Despite this, we did not find a relationship between prednisolone use, fractures or serum phosphorus levels. Assessment of bone turnover markers or histomorphometry from bone biopsies in future studies may provide informative insights of phosphorus metabolism in post-renal transplant bone disease. The use of calcitriol was not documented, and we acknowledge its potential effect on phosphorus metabolism and fracture risk. Urine phosphorus levels, TmP/GFR and FGF-23 levels were not available but would add valuable insights into the pathophysiology of the lower serum phosphorus levels. Longitudinal data of serum phosphorus levels may also provide important information with regards to future fracture risks, and this is the subject of a longitudinal study at our centre. Whether patients with lower serum phosphorus also have ongoing renal phosphate loss after transplantation, its relationship with FGF-23, and risk of fracture requires further evaluation in prospective studies.

Conclusion

Peripheral fractures affecting sites rich in cortical bone are common following renal transplantation and. currently, no consensus guidelines exist regarding optimal prevention, screening and management of bone health following renal transplantation. This is the first study to demonstrate that lower serum phosphorus levels post-renal transplantation had the strongest association with fracture. Although less predictive than phosphorus, monitoring of BMD at the FN may be useful in stratifying fracture risk for renal transplant recipients. Abnormalities in phosphorus homeostasis are common in renal transplant recipients and may contribute to adverse skeletal outcomes. Following this novel

1 finding, additional research examining the pathophysiology of phosphorus homeostasis and its
2 contribution to skeletal fragility following renal transplantation is now required.

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Table 1. Clinical, biochemical and BMD parameters of post-renal transplant recipients according to fracture status.

	Total (n=146)	No Fracture (n=106)	Fracture (n=40)	P value
<u>DEMOGRAPHICS</u>				
Age	53.9 ± 11.9	52.3 ± 11.3	58.2 ± 12.8	0.013*
Gender (Male/Female)	75/71	56/50	19/21	0.565
Menopausal (Y/N)	45/26	31/19	14/7	0.710
Weight (Kg)	75.8 ± 19.4	76.0 ± 19.5	74.9 ± 19.3	0.856
Height (cm)	166.2 ± 13.4	166.9 ± 14.5	164.3 ± 9.8	0.498
BMI [†]	27.4 ± 6.1	27.3 ± 6.3	27.5 ± 5.4	0.729
Total Dialysis Time (months)	31.7 (5.1-71.5)	29.0 (7.44-71.8)	35.2 (0.7-70.5)	0.867
SPK [#]	23	11	12	0.017*
Parathyroidectomy	11	9	2	0.476
Pre-existing Diabetes (%)	52 (36)	31 (29)	21 (53)	0.009*
Osteoporotic medication use	21	12	9	0.086
Prednisolone use (%)	126 (86)	89 (84)	37 (93)	0.181
Time post- Transplant (years)	5.1 (2.3- 9.3)	5.1 (2.3-9.3)	4.3 (2.4-9.2)	0.716
<u>BIOCHEMISTRY (reference ranges)</u>				
eGFR (>90 mls/min/1.73m ²)	51.80 ± 20.15	49.88 ± 19.99	57.02 ± 19.93	0.098
Creatinine (40-80 umol/L)	113.5 (89.5-158.5)	122.5 (93-168.3)	101.5 (74.5-139.5)	0.022*
PTH (1.50 -7.00 pmol/L)	8.5 (5.3-12.9)	8.1 (5.3- 11.6)	9.8 (6.0-13.8)	0.163
Vitamin D (75-250 nmol/L)	60.5 (43.2-75.9)	61.4 (43.2-61.4)	55.8 (40.9-72.9)	0.379
Calcium (2.20-2.60 mmol/L)	2.39 ± 0.16	2.377 ± 0.16	2.439 ± 0.15	0.076
Albumin (35-45 g/L)	39.21 ± 3.71	39.37 ± 3.91	38.800 ± 3.14	0.217
Adjusted Calcium (2.20-2.60 mmol/L)	2.40 ± 0.15	2.38 ± 0.15	2.45 ± 0.14	0.013*
Phosphorus (0.80-1.50 mmol/L)	1.10 ± 0.33	1.15 ± 0.37	0.996 ± 0.16	0.002*
ALP (30-110 U/L)	86.30 ± 51.50	86.01 ± 53.82	87.075 ± 45.46	0.517
Hb (110-160 g/L)	131.44 ± 18.27	130.08 ± 18.79	135.050 ± 16.49	0.166
TSH (0.30- 5.0 mU/L)	1.43 (0.94-2.00)	1.34 (0.94-2.00)	1.48 (0.90-2.11)	0.710
<u>BMD PARAMETERS</u>				
L1-L4 T-score	-0.50 (-1.50 to 0.50)	-0.50 (-1.50 to 0.50)	-0.60 (-1.58 to 0.58)	0.881
FN T-score	-1.60 (-2.30 to -0.90)	-1.40 (-2.00 to -0.70)	-1.90 (-2.68 to -1.25)	0.031*
TB T-score	-0.95 (-1.73 to 0.23)	-0.80 (-1.70 to 0.3)	-1.10 (-2.10 to -0.20)	0.124
L1-L4 Z-score	-0.20 (-1.10 to 0.90)	-0.20 (-1.1 to 0.9)	0.00 (-1.08 to 0.98)	0.422

FN Z-score	-0.80 (-1.40 to -0.20)	-0.70 (-1.35to -0.15)	-1.10 (-1.48 to -0.20)	0.173
TB Z-score	-0.55 (-1.40 to 0.28)	-0.40 (-1.40 to 0.40)	-0.70 (-1.50 to -0.20)	0.292
L1-L4 aBMD	1.148 (1.005 to 1.264)	1.154 (1.002 to 1.283)	1.137 (1.018 to 1.294)	0.906
FN aBMD	0.819 (0.744 to 0.936)	0.844 (0.751 to 0.951)	0.744 (0.714 to 0.890)	0.039*
TB aBMD	1.102 (1.027 to 1.196)	1.120 (1.027 to 1.221)	1.081 (1.027 to 1.155)	0.121

[†]BMI: body mass index, [#]SPK: simultaneous pancreas kidney transplant, aBMD: areal bone mineral density (g/cm²)

Data presented as mean ± SD when normally distributed or median and interquartile range (IQR 25-75) when not normally distributed.

* Denotes significant P value (p <0.05) between fracture and non-fracture patients

Conversion factors for units: creatinine in umol/L to mg/dL x0.0113, PTH in pmol/L to pg/ml x9.4, vitamin D in nmol/L to ng/ml x 0.401, calcium in mmol/L to mg/dL x 4, albumin: in g/L to g/dL x0.1, phosphorus; in mmol/L to mg/dL x3.1

Table 2. Characteristics and frequency of fractures in post-renal transplant recipients

	Males (n=75) (%)	All Females (n=71) (%)	Total (n)
No. of patients with fracture	26 (35)	26 (37)	52
No. of Fractures	43	36	79
Fracture type			
Feet/Toes	21	7	28
Ankle	1	9	10
Knee	1	2	3
Hand and wrist	6	8	14
Shoulder/arm	3	2	5
Rib	5	4	9
Vertebrae	3	2	5
Sternum	2	0	2
Hip	1	2	3
No. of patients with fracture			
Pre-transplant	7	5	12
Post-transplant	19	21	40

Table 3. Proportion of post-renal transplant recipients with osteopenia and osteoporosis range T scores or Z scores indicating low bone mass in pre-menopausal women

	Males n(%)	Post-menopausal females n(%)	Premenopausal females n(%)
Femoral Neck			
T $\leq$ -2.5	18(24)	8(17)	
T -1-2.5	34(45)	30(67)	
Z < -2.0			5(19)
Lumbar Spine			
T $\leq$ -2.5	9(12)	6(13)	
T -1-2.5	10(13)	19(42)	
Z < -2.0			4(15)
Total Body			
T $\leq$ -2.5	10(13)	6(13)	
T -1-2.5	24(32)	19(42)	
Z < -2.0			2(8)

Table 4. Multivariable regression analysis showing the association between prednisolone use and BMD in post-renal transplant recipients*

	β	P value
L1-L4 aBMD	-0.164	0.035
FN aBMD	-0.124	0.112
TB aBMD	-0.104	0.165

*Adjusted for age, gender and weight.

aBMD: areal bone mineral density (g/cm²)

Table 5. Multivariable logistic regression model displaying risk factors for fracture post-renal transplantation

	OR (95% CI)	p-value
Femoral neck aBMD[#]	0.059 (0.004-0.937)	0.045*
	OR (95% CI)	p-value
Femoral Neck T-score[#]	0.663 (0.447-0.985)	0.042*
	OR (95% CI)	p-value
Phosphorus[#]	0.053 (0.005-0.516)	0.011*

[#] Adjusted for age (per additional year increase), gender, eGFR (per additional mls/min/1.732 reduction), weight (per additional kg), pre-transplant fracture (presence/absence), FN aBMD (g/cm²), FN T score (per SD), phosphorus (per mmol/L change)

* Denotes significant P value (p <0.05)

Table 6. Multivariable logistic regression model displaying risk factors for fracture post-renal transplantation

	OR (95% CI)	p-value
Femoral Neck T-score	1.574 (0.983-2.526)	0.059
Phosphorus	2.620 (1.210-5.746)	0.015*

#Adjusted for age (per additional year increase), gender, eGFR (per additional mls/min/1.732 reduction), weight (per additional kg), pre-transplant fracture (presence/absence).

OR expressed per SD decline.

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