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ORIGINAL PAPER

Impact of kidney transplantation on serum bone mineral levels and anemia – a cohort study on Egyptian children

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ABSTRACT

Introduction: Disorder of bone mineral indicators and anemia are frequent morbidities in children with end stage kidney disease (ESKD) that can persist after kidney transplantation (KT). This study aims to investigate the effect of KT on bone minerals and anemia by comparing their levels before KT and at regular follow-up intervals after KT.

Material and methods: A cohort of 30 pediatric kidney transplant recipients (KTRs) was followed up at 3-month intervals for their first post-transplantation year. Review of pre-transplantation, transplantation-related data and regular measurement of serum calcium, phosphorus, alkaline phosphatase (ALP), parathyroid hormone (PTH), hemoglobin, hematocrit, iron and ferritin was performed.

Results: Serum phosphorus and PTH levels significantly decreased after KT ($p < 0.001$) with no significant change in calcium ($p = 0.221$) or ALP ($p = 0.377$) levels. Frequency of hyperparathyroidism significantly decreased after KT ($p < 0.05$) but 53.3% and 63.3% of patients had hyperparathyroidism at 6 and 12 months respectively. Parathyroid hormone did not show a significant difference at 6- and 12-month assessments after transplantation ($p = 0.82$). Hemoglobin level, hematocrit and serum iron significantly increased after KT ($p = 0.019$, 0.048 and 0.008 respectively). Frequency of phosphorus level abnormalities and anemia significantly declined after KT ($p < 0.001$ and 0.0356 respectively). Parathyroid hormone levels positively correlated with glomerular filtration rate ($p = 0.004$ and CC = 0.608). Patients on tacrolimus had less phosphorus than those on cyclosporine at 12 months ($p = 0.005$).

Conclusions: Successful KT in children partially normalizes bone mineral disorders accompanied with ESKD by reducing serum phosphorus levels. Hyperparathyroidism is prevalent by the end of the first post-transplantation year. Anemia does still exist after KT but to a lesser extent than pre-transplantation.

KEY WORDS:

anemia, children, kidney transplantation, phosphorus, PTH.

INTRODUCTION

Disordered regulation of bone mineral metabolism is a common, well-known alteration occurring in the course of chronic kidney disease (CKD) in children [1].

Alterations in bone modeling/remodeling or vascular biology in pediatric CKD children, as a consequence of disordered regulation of mineral metabolism, severely impact their quality of life as well as life span [2].

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The abrupt change in kidney function and the medications required to maintain a graft are competing forces that simultaneously affect bone disease in pediatric kidney transplant recipients (KTRs) [3]. Moreover; hyperparathyroidism has been reported even in patients with a normal glomerular filtration rate (GFR), which indicates that other factors in addition to kidney function are involved in this defect [4].

Disorders of calcium metabolism were reported to be partially or completely corrected after successful kidney transplantation (KT) [5]. Nevertheless, nutritional vitamin D deficiency, persistent hyperparathyroidism, tertiary FGF-23 excess, hypophosphatemia, and immunosuppressive therapy continue to impair bone health and growth [6]. Evaluation and treatment of mineral bone homeostasis are still not standardized, mainly due to the numerous factors impacting it [3].

Although successful KT may correct anemia, up to 20–51% of patients remain anemic after transplantation at various time points [7]. Post-transplantation anemia (PTA) is either early (within 6 months after KT), which is usually associated with surgical blood losses and iron deficiency, or late (> 6 months after KT), which results from graft dysfunction; however, iron deficiency, drug toxicity, and post-transplant inflammation also play a role [8].

It is important to determine the pattern and outcome of bone mineral disorders and anemia before and after KT and to describe their prevalence in pediatric KTRs. The aim of this study is to investigate the effect of KT and immunosuppressive therapy on bone minerals and anemia in children by comparing their levels before KT and at regular follow-up intervals after KT until the end of the first post-transplantation year.

MATERIAL AND METHODS

This is a longitudinal cohort study that included 30 pediatric KTRs. Included patients were children with end stage kidney disease (ESKD) who underwent KT during the study period. Patients were recruited from the Kidney Transplantation Outpatient Clinic, Cairo University Children's Hospital during their follow-up immediately after transplantation. The study was conducted over 17 months during the period between 2013 and 2015.

The study protocol was approved by the Faculty of Medicine, Cairo University (N: I-010313). Informed consent for enrolment in the study was obtained from patients' legal guardians. All procedures used in this study were in adherence to the Declaration of Helsinki 1967.

Eligible patients aged 2–16 years were included in the study. All included patients were recipients of a living (according to national regulations) kidney transplant for their first time. Included patients had their KT operation performed during the study period at the Kidney Transplantation Unit, Cairo University Children's Hospital, Cairo, Egypt. Kidney transplant recipients with incomplete medical records or those who were noncompliant to follow-up were excluded from the study.

A total of thirty freshly transplanted included patients were subjected to clinical assessment, retrospective analysis, review of transplantation-related data and prospective analysis.

CLINICAL ASSESSMENT

History of baseline demographic data in the form of age and sex was taken. Clinical examination included anthropometric measurements, vital signs, and systematic examination. Height and weight of the patients were obtained at initial assessment and body mass index was calculated. Systolic and diastolic blood pressure (BP) values were expressed as a ratio to the 90th percentile for age and sex (BP index) for standardization. Blood pressure was categorized into: normal BP: < 90th percentile, elevated BP: ≥ 90th percentile to < 95th percentile or 120/80 mm Hg to < 95th percentile (whichever is lower), stage 1 hypertension (HTN): ≥ 95th percentile to < 95th percentile + 12 mm Hg, or 130/80 to 139/89 mm Hg (whichever is lower), stage 2 HTN: ≥ 95th percentile + 12 mm Hg, or ≥ 140/90 mm Hg (whichever is lower) [9].

RETROSPECTIVE ANALYSIS OF PRE-TRANSPLANTATION DATA

This was done by reviewing medical records of included patients while they were prepared for transplantation (pre-transplantation data). Pre-transplantation medical history (onset of renal dialysis – original kidney disease, dialysis duration, and any major pathological conditions (e.g. hepatitis C infection)) was taken. Review of pre-transplantation laboratory (serum levels of blood urea nitrogen), creatinine, calcium, phosphorus, alkaline phosphatase (ALP) parathyroid hormone (PTH), hemoglobin (Hb), and hematocrit and serum iron) investigations was performed. All laboratory indicators were evaluated pre-dialysis (48 hours after the previous dialysis session). Review of erythropoietin and iron administration before KT was also performed. Erythropoietin is usually given in a dose of 100–200 units/kg three times weekly subcutaneously targeting Hb > 10 g% and hematocrit of 35–40%.

REVIEW OF TRANSPLANTATION-RELATED DATA

Data were in the form of age at transplantation, donor age/relation, virology status at the time of KT (CMV IgG and HCV ab), immunosuppressive medications received during the procedure, and transplantation-related morbidities mainly in the form of infections and acute rejection episodes. Patients with calcium/phosphorus disorders after KT are usually treated with a nutritional regimen (encouraging adequate vitamin D and calcium rich

diet, phosphorous restriction usually no longer needed after KT, increasing phosphorous intake is advised early after transplantation in hypophosphatemia). Supplements (in the form of vitamin D, oral phosphorus or calcium) may be needed in patients not responsive to the nutritional regimen only or severe calcium/phosphorus disorders.

PROSPECTIVE ASSESSMENT OF POST-TRANSPLANTATION FOLLOW-UP

All included patients were followed up for at least 1 year after KT at 3-month intervals. In addition to the routine assessment of serum creatinine at their regular follow-up visits, follow-up serum levels of calcium, phosphorus, ALP, Hb and hematocrit were assessed at 3, 6, 9 and 12 months after transplantation. Serum ferritin and PTH were analyzed at 6 and 12 months after transplantation.

DEFINITIONS

Serum calcium levels were considered normal if 9.4–10.8 mg/dl at 2–5 years, 9.4–10.3 mg/dl at 6–12 years and 9.1–10.2 mg/dl > 12 years [10]. Serum phosphorus levels were considered normal if 3.8–6.51 mg/dl at 2–3 years, 3.7–5.6 mg/dl at 4–11 years, 2.9–5.4 mg/dl at 12–15 years and 2.7–4.7 mg/dl > 15 years [11]. Normal PTH levels were considered 14–65 pg/ml [12]. Anemia was defined as Hb level < 10.9 g/dl under 5 years, < 11.9 at 5–11 years and < 11.9 in females 11–18 years and 12.7 in males 11–18 years [13].

STATISTICAL ANALYSIS

Data were analyzed using IBM SPSS Advanced Statistics, version 21. Data were checked for normal distribution by means of the Kolmogorov-Smirnov test and Shapiro-Wilk test. Numerical data were described as mean and standard deviation or median and range, as appropriate. Categorical data were summarized as percentages. In compliance with the distribution of data, Student's *t*-test or χ^2 test was used for comparisons between groups. Intragroup comparisons were performed using parametric or non-parametric repeated measures analysis of variance (ANOVA) or Friedman test as appropriate. Bivariate correlation analysis was performed for association between continuous variables. The χ^2 test or Fisher's exact test was used to compare between the groups with respect to categorical data. All tests are two tailed. A *p*-value less than or equal to 0.05 was considered statistically significant

RESULTS

The mean age of the study group was 12.5 ±3.5 years with a male to female ratio of 3.2/1 [23 (76.7%) males and 7 (23%) females]. One patient received no antibody induction therapy due to low immunological risk (zero human leukocyte antigen mismatch) while 29 patients received either antithymocyte globulin (ATG) or basiliximab. The patient who did not receive antibody induction immunosuppression therapy was excluded from analysis of data related to anemia due to the potential impact on erythropoiesis. The maintenance immunosuppression protocol, according to the center protocol, consisted of steroids, calcineurin inhibitors (CNI) – either cyclosporine or tacrolimus – and adjuvant therapy in the form of mycophenolate mofetil. Cyclosporine was the main CNI used initially after KT (90%) and tacrolimus was used upon individual indications. Patients were shifted from cyclosporine to tacrolimus during their follow-up visits according to their clinical status so that by the end of the study only 43.3% were still on the cyclosporine-based triple immunotherapy protocol. Steroids were given in high intravenous doses during induction of immunosuppression then tapered gradually to oral steroids targeting a dose of 5–7.5 mg by 6 months and 2.5–5 mg by the end of the first post-transplantation year. Pulse methylprednisolone therapy was the first line antirejection therapy that was given for all patients who experienced acute rejection episodes with further management of rejection depending on the pathological diagnosis (ATG in T-cell mediated rejection and plasma exchange/intravenous immunoglobulin and rituximab in antibody mediated rejection). Clinical, pre-transplantation, and transplantation related data of included patients are summarized in Table 1.

Serum phosphorus and PTH levels significantly decreased after KT ($p < 0.001$) with no significant change in serum calcium levels ($p = 0.221$) or ALP ($p = 0.377$). Parathyroid hormone showed no significant difference in its levels at 6 and 12 months ($p = 0.82$). Hemoglobin level, hematocrit and serum iron significantly increased after KT ($p = 0.019$, 0.048 and 0.008 respectively) (Table 2). Frequency of abnormal levels of serum calcium, phosphorus, ALP, PTH and Hb are illustrated in Table 3. Erythropoietin and iron therapy were needed in a frequency of 73.3% and 80% respectively before KT. After KT erythropoietin and iron therapy were needed mainly during the early period after the operation with frequency of administration at 3, 6 and 12 months being 3.3%, 3.3% and 0% for erythropoietin and 36.7%, 23.3% and 13.3% for iron respectively. Vitamin D therapy at 3, 6, 9 and 12 months after KT was needed in a frequency of 30%, 33.3%, 20% and 16.7% respectively, mainly to correct hypophosphatemia. Angiotensin converting enzyme inhibitors were not administered to any of the included patients during the first post-transplantation year.

Frequency of phosphorus level abnormalities and anemia at the 12-month follow-up after KT significantly decreased when compared to pre-transplantation frequencies ($p < 0.001$ and 0.0356 respectively). Frequency of PTH abnormalities significantly decreased after

TABLE 1. Clinical, pre-transplantation and transplantation-related data of the study group ($n = 30$)

Parameters	Ratio	Value
Weight [kg]	Mean ±SD	30 ±12.9
Height [cm]	Mean ±SD	138 ±17.9
BMI [kg/m^2]	Mean ±SD	19.24 ±6.19
BP pre-TX (normal, elevated, stage 1 HTN, stage 2 HTN) BP at 3 months (normal, elevated, stage 1 HTN, stage 2 HTN) BP at 6 months (normal, elevated, stage 1 HTN, stage 2 HTN) BP at 9 months (normal, elevated, stage 1 HTN, stage 2 HTN) BP at 12 months (normal, elevated, stage 1 HTN, stage 2 HTN)	*n* (%)	7 (23.3), 6 (20), 9 (30), 8 (26.7) 5 (16.7), 14 (46.7), 9 (30), 2 (6.7) 6 (20), 10 (33.3), 13 (43.3), 1(3.3) 5 (16.7), 20 (66.7), 5 (16.7%), 0 (0) 12 (40), 16 (53.3), 2 (6.7), (0)
Original kidney disease*	*n* (%)	
Glomerular disease		10 (33.3)
Unknown (bilateral atrophic kidneys)		9 (30)
Obstructive uropathy		5 (16.7)
Tubulointerstitial diseases		5 (16.7)
Vascular diseases		1 (3.3)
Dialysis duration (months)	Mean ± SD Median (range)	22.6 ±28.7 8.5 (0–132)
Age at transplantation (years)	Mean ± SD	11.29 ± 3.49
Donor age (years)	Mean ± SD	36 ±7
Donor relation	*n* (%)	
Related		26 (86.7)
Unrelated		4 (13.3)
Virology status	*n* (%)	
CMV IgG +ve		29 (96.7)
CMV IgG –ve		1 (3.3)
HCV Ab +ve		7 (23.3)
HCV Ab –ve		23 (76.7)
Antibody induction immunosuppression	*n* (%)	
None		1 (3.33)
ATG		23 (76.7)
Basiliximab		6 (20)
Maintenance CNI immunosuppression		
1st month (C/T)		27/3
3 months (C/T)		23/7
6 months (C/T)		20/10
9 months (C/T)		13/17
12 months (C/T)		13/17
Daily steroid dose after TX [mg/day]	Mean ± SD	
1st month		32.67 ±5.83
3 months		13.67 ±5.48
6 months		11.67 ±6.74
9 months		9.5 ±3.62
12 months		7.32 ±4.02
Post-transplantation infection	*n* (%)	
GIT infections		18 (60)
UT infections		18 (60)
Post-transplantation acute rejection episodes	Mean ± SD Median (range)	2.13 ±1.4 2.5 (0–6)

ATG – antithymocyte globulin, BMI – body mass index, BP – blood pressure, C – cyclosporine, CMV – cytomegalovirus, CNI – calcineurin inhibitors, GIT – gastrointestinal tract, HCV – hepatitis C virus, HTN – hypertension, T – tacrolimus, TX – transplantation, UT – urinary tract

** Glomerular diseases includes focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, lupus nephritis and crescentic glomerulonephritis. Tubulointerstitial diseases include hereditary nephronophthisis and chronic tubulointerstitial nephritis. Obstructive uropathy incudes vesicoureteric reflux, posterior urethral valve and neurogenic bladder. Vascular lesion is antiphospholipid syndrome.*

TABLE 2. Follow-up assessment of laboratory data of the study group

Parameters	Pre-TX Mean ±SD	3 months after TX Mean ±SD	6 months after TX Mean ±SD	9 months after TX Mean ±SD	12 months after TX Mean ±SD	*p*-value
BUN [mg/dl]	59.7 ±44	21.2 ±8	20.2 ±7	23.0 ±13.4	20.9 ±15.8	< 0.001
Serum creatinine [mg/dl]	5.7 ± 1.8	0.75 ±0.24	0.90 ±0.32	0.86 ± 0.26	0.91 ± 0.44	< 0.001
GFR [ml/min/1.73 m^2]	14.0 ± 8.6	92.6 ±31.5	87.4 ±44.5	86.1 ±25.18	75.3 ±32.3	< 0.001
Serum calcium [mg/dl]	9.5 ±1.8	9.7 ±0.9	9.7 ±0.7	9.6±0.8	9.8 ±0.5	0.221
Serum phosphorus [mg/dl]	6.08 ± 1.7	4.0 ±0.74	4.01 ± 0.93	4.15±1.06	4.28 ±0.74	< 0.001
ALP [IU/l] Median (range)	323 ±274 239.5 (64–1465)		254 ±110 226.5 (105–584)		249 ±100 223.5 (79–486)	0.377
PTH [pg/ml] Median (range)	489.5 ±547.9 271 (52–1970)		82.1 ±82.7 57.1 (17– 431)		94.7 ±98.7 69.3 (16.1–509)	< 0.001
Hemoglobin [gm/dl]	10.4 ±1.57	11.12 ±1.5	11.43 ±1.46	11.63 ±1.38	11.57 ±1.89	0.019
Hematocrit (%)	31.73 ±4.3	34.24 ±4.7	33.96 ±4.13	34.62 ±3.95	34.54 ±3.35	0.048
Serum iron [µg/dl]	65.9 ±25	75.78 ±31	76.98 ±19.91	87.45 ±25.3	86.3 ±22.96	0.008
Ferritin [ng/ml]			877.3 ±1331.6		436.3 ±405.4	0.094

ALP – alkaline phosphatase, BUN – blood urea nitrogen, GFR – glomerular filtration rate, PTH – parathyroid hormone, TX – transplantation

TABLE 3. Incidence of hyperparathyroidism ($n = 30$) and anemia ($n = 29$) after transplantation

Parameters		Pre-TX, *n* (%)	Pre-TX vs. 6 months after TX, *p*-value	Pre-TX vs. 12 months after TX, *p*-value	At 6 months after TX, *n* (%)	At 12 months after TX, *n* (%)	3 months vs. 6 months after TX, *p*-value
Calcium	Normal	22 (73.3)	1	1	22 (73.3)	22 (73.3)	1
	Hyper	8 (26.7)			8 (26.7)	8 (26.7)	
Phosphorus	Normal	17 (56.7)	<0.0001	<0.0001	19 (63.3)	26 (86.7)	0.736
	Hypo	0 (0)			11 (36.7)	4 (13.3)	
	Hyper	13 (43.3)			0 (0)	0 (0)	
ALP	Normal	22 (73.3)	0.095	0.347	27 (90)	25 (83.3)	0.448
	Hyper	8 (26.7)			3 (10)	5 (16.7)	
PTH	Normal	2 (6.7)	0.00132	0.0122	14 (46.7)	11 (36.7)	0.6004
	Hyper	28 (93.3)			16 (53.3)	19 (63.3)	
Anemia		19 (65.5)	0.0659	0.0356	11 (37.9)	10 (34.5)	1

ALP – alkaline phosphatase, PTH – parathyroid hormone, TX – transplantation

transplantation both at 6-month ($p = 0.00132$) and at 12-month ($p = 0.0122$) assessments. No significant difference was detected in frequency of hyperparathyroidism or anemia before transplantation, at 6 and at 12 months between patients transplanted pre-emptively ($n = 5$) and those transplanted after dialysis (for hyperparathyroidism; 80% vs. 92% pre-transplantation, 40% vs. 56% at 6 months and 60% vs. 64% at 12 months with $p = 0.743$, 0.869 and 0.735 respectively, for anemia; 60% vs. 66.7% pre-transplantation, 40% vs. 37.5% at 6 months and 60% vs. 29.2% at 12 months with $p = 0.817$, 0.688 and 0.422 respectively).

As illustrated in Table 4, pre-transplantation serum ALP levels significantly correlated with dialysis duration ($p = 0.014$ and CC = 0.452). Parathyroid hormone levels at the 12-month follow-up significantly positively correlated with GFR at 6 months ($p = 0.004$ and CC = 0.608), but the negative correlation with number of acute rejection episodes, which reflects the frequency of pulse methyl prednisone therapy, was not significant ($p = 0.055$ and CC = –0.353). No significant correlation was detected between serum calcium, phosphorus, ALP or Hb levels and any of dialysis duration, serum creatinine, GFR, acute rejection episodes or oral daily steroid doses.

TABLE 4. Correlation between indicators of bone metabolism and different variables among the study group

Parameters	Formula	Calcium ($n = 30$)		Phosphorus ($n = 30$)		ALP ($n = 30$)		PTH ($n = 30$)		HbPost-TX at 6 m ($n = 29$)	Ht Post-TX at 6 m ($n = 29$)
		Pre-TX	Post-TX 6 m	Pre-TX	Post-TX at 6 m	Pre-TX	Post-TX at 6 m	Pre-TX	Post-TX at 12 m		
Dialysis duration (months)	*p*-value	0.707	0.684	0.183	0.070	0.014	0.458	0.866	0.815	0.425	0.559
	CC	–0.073	–0.080	–0.255	–0.342	0.452	0.144	0.032	–0.04	0.154	0.113
Age at TX (years)	*p*-value	0.689	0.286	0.085	0.476	0.870	0.657	0.198	0.331	0.756	0.448
	CC	0.076	–0.201	–0.319	–0.135	0.031	–0.084	0.242	0.184	–0.060	–0.146
Serum creatinine [mg/dl] at 6 m	*p*-value	0.442	0.181	0.733	0.451	0.171	0.564	0.197	0.164	0.253	0.263
	CC	0.146	–0.250	–0.065	–0.142	–0.256	0.109	–0.241	–0.26	–0.219	–0.215
GFR at 6 m [ml/min/1.73 m^2]	*p*-value	0.602	0.780	0.752	0.196	0.519	0.862	0.331	0.004	0.514	0.587
	CC	0.099	0.053	–0.059	0.242	0.122	0.023	0.183	0.608	0.126	0.105
Acute rejection episodes after TX (n)	*p*-value	–	0.246	–	0.672	–	0.666	0.940	0.055	0.246	0.585
	CC	–	–0.218	–	–0.080	–	–0.082	0.014	–0.353	–0.222	–0.105
Oral daily 6 m steroid dose [mg/day]	*p*-value	–	0.485	–	0.073	–	0.334	0.710	0.502	0.833	0.974
	CC	–	0.133	–	–0.331	–	–0.183	–0.070	–0.127	0.041	–0.006

ALP – alkaline phosphatase, GFR – glomerular filtration rate, Hb – hemoglobin, Ht – hematocrit, PTH – parathyroid hormone, TX – transplantation

By comparison between patients who received cyclosporine and those who received tacrolimus at different follow-up interval points after KT, no significant association was found between CNI type and any of serum calcium, ALP or PTH levels. Serum phosphorus levels were significantly lower (4 ±0.5 vs. 4.7 ±0.7, $p = 0.005$) in patients who received tacrolimus than cyclosporine (Table 5).

No significant difference was detected in measured indicators of bone mineral abnormalities and Hb levels between patients with different etiologies of renal illness (Table 6).

DISCUSSION

Although KT corrects or improves many complications of CKD, its effect on disordered mineral metabolism is not completely understood [14], and PTA is not uncommon [15]. The present study aimed to investigate the impact of KT and immunosuppressive therapy on bone minerals and anemia in pediatric ESKD patients.

The study showed significantly reduced serum phosphorus and PTH levels after KT with no significant change in serum calcium or ALP with prevalence of hyperparathyroidism at 12 months after KT (63.3%) and significant reduction in frequency of phosphorus and PTH abnormalities. The study also showed that Hb level hematocrit and serum iron significantly increased with significant reduction in frequency of anemia after KT when compared to pre-transplantation values. Parathyroid hormone levels at the 12-month follow-up significantly positively correlated with GFR at 6 months. None of the measured indicators of mineral bone abnormality correlated with graft function (in term of serum creatinine), acute rejection episodes or daily steroid doses. Serum phosphorus levels were significantly lower in patients who received tacrolimus than cyclosporine at 12 months.

Our results documented a direct positive impact of KT on PTH levels by observing significantly lower levels of PTH ($p < 0.001$) and lower frequency of hyperparathyroidism both at 6 months ($p = 0.001$) and 12 months ($p = 0.012$) after transplantation. Nevertheless, we noted that 53.3% of patients had normal parathyroid functions at 6 months and 63.3% at 12 months after KT. With successful KT and higher GFR, most of the stimuli of parathyroid hyperplasia abate. This often leads to a gradual decline in PTH concentrations. Unlike FGF-23 levels that precipitously decline after KT, the fall in PTH is more gradual. It has been reported that 25 to > 80% of patients still have inappropriately high PTH beyond 1 year after transplantation [14, 16]. Estimated GFR of the present study ensures successful KT of included patients. Even the noted decline of GFR with follow-up that is partially explained by occurrence of repeated acute rejection ep-

TABLE 5. Comparison between indicators of mineral bone disease, hemoglobin and hematocrit in patients receiving cyclosporine and tacrolimus

Parameters	Cyclosporine group		Tacrolimus group		*p*-value
	n	Mean ±SD	*n*	Mean ±SD	
Serum calcium level					
At 3 months	23	9.6 ±0.8	7	10 ±1.2	0.439
At 6 months	20	9.6 ±0.6	10	10 ±0.9	0.146
At 9 months	13	9.4 ±0.76	17	9.7 ±0.87	0.310
At 12 months	13	9.6 ±0.7	17	9.9 ±0.7	0.101
Serum phosphorus level					
At 3 months	23	4 ±0.7	7	3.9 ±0.9	0.851
At 6 months	20	4 ±0.9	10	3.8 ±0.7	0.717
At 9 months	13	4.1 ±1.1	17	4.1 ±1	0.948
At 12 months	13	4.7 ±0.7	17	4 ±0.5	0.005
Serum ALP level					
At 3 months	23	243.3 ±127	7	207 ±150	0.615
At 6 months	20	267 ± 144.4	10	212 ±89	0.262
At 9 months	13	277.5 ±127	17	243 ±161	0.518
At 12 months	13	281.8 ±110	17	230 ±92	0.186
PTH level					
At 6 months	20	84.15 ± 92.11	10	78.3 ±64.36	0.859
Median (range)		59.99 (17–431.2)		52.48 (20–222)	
At 12 months	13	82.73 ±71.72	17	105.16 ±114.31	0.5409
Median (range)		62.8 (23.8–293.5)		75 (16.1–509)	
Hb level					
At 3 months	22	10.91 ±1.51	7	11 ±2.1	0.915
At 6 months	19	11.47 ±1.66	10	11.3 ±1.1	0.774
At 9 months	12	11.48 ±1.42	17	11.2 ±1.4	0.602
At 12 months	12	11.44 ±1.24	17	11.6 ±1.1	0.717
Ht					
At 3 months	22	33.59 ±4.32	7	34 ±6.1	0.845
At 6 months	19	34.25 ±4.68	10	33 ±2.8	0.447
At 9 months	12	34.77 ±3.65	17	34.6 ±3.7	0.903
At 12 months	12	34.88 ±3.95	17	36 ±2.7	0.371

ALP – alkaline phosphatase, Hb –hemoglobin, Ht – hematocrit, PTH – parathyroid hormone

isodes in some of the patients is still well acceptable as compared to international standard [17, 18].

Similar results were also reported by Sprague *et al*. They reviewed the literature between January 1990 and October, 2006 and they found that PTH levels decreased significantly during the first 3 months after KT but typically stabilized at elevated values after 1 year [19]. Moreover, in up to 50% of patients there is evidence of a persistent elevation in PTH years after a successful KT [20]. These data support the fact that hyperparathyroidism remains a common problem among KTRs and that the risk of bone disease and its complications varies throughout the post-transplant timeline.

Our results showed a prevalence of 27.7% of hypercalcemia after KT (at 6- and 12-month follow-up). Hypercalcemia has been reported in 11–31% of KTRs within 1 year [14, 21, 22], with some studies noting prevalence of more than 50% of patients, especially in those who had moderate to severe hyperparathyroidism prior to KT [3]. Post-transplantation hypercalcemia is multifactorial; most patients have an inappropriately high PTH level where no further work-up may be needed. However, if PTH is appropriately suppressed, evaluation of hypercalcemia as in non-transplant patients should be performed [23].

The present study reported a significant decline in serum phosphorus level after KT although it was not mea-

TABLE 6. Comparison between indicators of bone mineral abnormalities and anemia in different etiologies of renal diseases

Parameters	Glomerular diseases ($n = 10$)	Unknown ESKD ($n = 9$)	Obstructive uropathy ($n = 5$)	Tubulointerstitial diseases ($n = 5$)	Vascular diseases ($n = 1$)	*p*-value
Pre-TX Ca	8.7 ±1.6	9.37 ±1.3	9.6 ±1.6	11.1 ±2.65	10.2	0.191
Ca at 12 months	9.97 ±0.68	9.62 ±0.55	9.97 ±0.68	9.8 ±0.39	10.6	0.490
Pre-TX Po4	5.9 ±1.1	6.46 ±1.9	5.4 ±0.94	6.67 ±2.89	5.2	0.716
Po4 at 12 months	4.2 ±0.77	4.37 ±0.67	3.8 ±0.31	4.48 ±0.51	4.5	0.479
Pre-TX ALP	342.9 ±414	314.9 ±156.9	354.5 ±300.8	263.2 ±131.3	300	0.988
ALP at 12 months	244.8 ±114.7	252.3 ±77.4	222.4 ±152.7	285.6 ±72.4	198	0.880
Pre-TX PTH	297.5 ±262	463.3 ±610.8	596.6 ±533.7	835 ±854.6	385	0.508
PTH at 12 months	57.4 ±29.9	120.3 ±155.5	108.4 ±105	123.1 ±23.6	50	0.616
Pre-TX HB	9.8 ±0.99	9.9 ±1.5	11.2 ±1.7	11.08 ±1.5	9.2	0.204
Hb at 12 months	11.5 ±1.2	11.5 ±1.2	11.9 ±1.3	11.64 ±1.4	10.8	0.963

ALP – alkaline phosphatase, Ca – calcium, ESKD – end stage kidney disease, GFR –glomerular filtration rate, Hb –hemoglobin, Po4 – phosphorus, PTH – parathyroid hormone

sured immediately after surgery but at 3 months after KT. Nevertheless, levels of phosphorus had a stable pattern all through follow-up intervals during the first post-transplantation year. Our prevalence of hypophosphatemia declined from 36.7% at 6 months to 13.3% at the 12-month follow-up. Hypophosphatemia is common in KTRs and was reported to occur in up to 50% of patients mostly 3–4 weeks after KT, particularly in KTRs, with immediate excellent graft function [3]. Phosphorus levels usually begin to normalize within the first few months, correlating with the decline in FGF-23 levels [14], unless patients have persistent hyperparathyroidism where renal phosphorus wasting persists for months after KT.

Similar results were reported by Bonthuis *et al*., in a study that included 1237 children from 10 European countries. They found that 25% of patients had abnormal serum phosphorus. A longer time since transplantation was associated with a lower risk of having mineral levels above the target range [22].

Calcium metabolism after KT was also investigated by Evenepoel *et al*. They monitored calcium, phosphorus, PTH and calcitriol in 201 KTRs at the time of KT and 3 months thereafter. They found that serum calcium levels followed a biphasic pattern with a significant decline during the first postoperative week, followed by a significant increase. High pre-transplantation PTH levels protect against hypocalcemia within the first postoperative week but put patients at risk for late hypercalcemia [24].

The results of this study showed that PTH levels at 12 months significantly correlated with GFR at 6 months. Although persisting hyperparathyroidism after KT can lead to worsening graft function, bone disease and extra-skeletal calcifications [25], concerns have been raised that parathyroidectomy adversely affects the graft. A decrease in creatinine clearance by 10% was reported, without relevant recovery during a 12-month follow-up in the retrospective study of Schwarz *et al.* [26]. Evenepoel *et al*. reported an increase in serum creatinine by 16% in the first 6 months after parathyroidectomy, with partial reversal and stabilization of graft function in the long term over 4 years [27]. The impact of persistent hyperparathyroidism on graft outcome is still debated [28]. However, it seems that physiological levels of PTH are necessary to maintain adequate graft function after KT.

Although our study showed significant reduction in frequency of anemia after KT, as anticipated, prevalence of PTA was still high (37.9% at 6 months and 34.5% at 1 year). The prevalence of early PTA was reported to be up to 50% [7] while late PTA declines to about 23–35% at various time points up until 8 years after KT [15, 29]. The most commonly reported causes of late PTA were nutritional deficiencies, which accounted for 61% of cases, of which iron deficiency was diagnosed in 34.7% of cases [29]. Early PTA has been shown to be a predictor of late PTA, and the occurrence of late PTA has been associated with impaired graft function [15].

Glucocorticoids can reduce the expression of Na-Pi cotransporters and worsen urinary loss of phosphorus after KT and can also reduce oral phosphorus absorption in the intestines [3]. Nevertheless, the present study failed to outline a significant direct association between either daily steroid doses or pulse steroid therapy or any parameters of bone metabolism, Hb level or hematocrit percentage.

The study revealed lower serum phosphorus levels in KTRs who received tacrolimus than cyclosporine at 12 months. This is not in line with the previously reported data suggesting that tacrolimus-based immunosuppression leads to faster recovery from tubular phosphate reabsorption impairment [30]. The minute difference of phosphorus levels and the long interval after transplant surgery limit our results in this regard.

This study is limited by the small sample size, relatively short duration of follow-up, lack of concomitant vitamin D and FGF-23 levels and lack of detailed data on

therapeutic interventions for mineral bone abnormalities. Further studied are highly recommended to overcome these limitations.

CONCLUSIONS

Successful KT in children could partially normalize bone mineral disorders accompanied with CKD by reducing serum phosphorus and reducing the frequency of phosphorus level abnormalities. Hyperparathyroidism does still occur after KT and should be detected and probably managed. KT reduces anemia accompanied by CKD; nevertheless, PTA still exists albeit with lower frequency. The study did not reveal a direct impact of immunosuppressive drugs received after KT on measured indicators of bone mineral abnormalities and the lower phosphorus level noted among tacrolimus patients needs to be validated by other studies with a larger sample size.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Wesseling-Perry K, Salusky IB. Chronic kidney disease: mineral and bone disorder in children. Semin Nephrol 2013; 33: 169-179.
2. Wesseling-Perry K, Salusky IB. Mineral and bone disorders in children with chronic kidney disease. In: Avner E, Harmon W, Niaudet P, et al. (eds.). Pediatric Nephrology. Springer, Berlin, Heidelberg 2016.
3. Vangala C, Pan J, Cotton RT, Ramanathan V. Mineral and bone disorders after kidney transplantation. Front Med 2013; 5: 211.
4. Díaz-Barriga D, Hernández-Sánchez AM, Rico-Argüello Y, et al. Pre- and post-renal transplant bone mineral metabolism in children and adolescents. Bol Med Hosp Infant Mex 2013; 70: 114-120.
5. Alshayeb HM, Josephson MA, Sprague SM. CKD-mineral and bone disorder management in kidney transplant recipients. Am J Kidney Dis 2013; 61: 310-325.
6. Sgambat K, Moudgil A. Optimization of bone health in children before and after renal transplantation: current perspectives and future directions. Front Pediat 2014; 2: 13
7. Gafter-Gvili A, Cohen E, Avni T, et al. Predicting the emergence of anemia – a large cohort study. Eur J Intern Med 2015; 26: 338-343.
8. Kouri A, Balani S, Kizilbash S. Anemia in pediatric kidney transplant recipients –etiologies and management. Front Pediatr 2020; 10: 929504.
9. Flynn JT, Kaelber DC, Baker-Smith CM, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. Pediatrics 2017; 140: e20171904.
10. Lietman SA, Germain-Lee EL, Levine MA. Hypercalcemia in children and adolescents. Curr Opin Pediatr 2010; 22: 508-515.
11. Lockitch G, Halstead AC, Albersheim S, MacCallum C, Quigley G. Age- and sex-specific pediatric reference intervals for biochemistry analytes as measured with the Ektachem-700 analyzer. Clin Chem 1988; 34: 1622-1625.
12. Available from: https://www.hyperparathyroidmd.com/normal-range-pth-blood-test/.
13. Available from: https://www.healthline.com/health/normal-hemoglobin-levels.
14. Wolf M, Weir M, Kopyt N, et al. A prospective cohort study of mineral metabolism after kidney transplantation. Transplantation 2016; 100: 184-193.
15. Gafter-Gvilia A, Gafter U. Post-transplantation anemia in kidney transplant recipients. Acta Haematol 2019; 142: 37-43.
16. Egbuna OI, Taylor JG, Bushinsky DA, Zand MS. Elevated calcium phosphate product after renal transplantation is a risk factor for graft failure. Clin Transplant 2007; 21: 558-566.
17. Bucsa C, Stefan G, Tacu D, et al. Does the KDIGO CKD risk stratification based on GFR and proteinuria predict kidney graft failure? Int Urol Nephrol 2014; 46: 1857-1865.
18. Beak CH, Kim H, Yang WS, Han DJ, Park S. A postoperative 1-year eGFR of more than 45 ml/min may be the cutoff level for a favorable long-term prognosis in renal transplant patients. Ann Transplant 2016; 21: 439-447.
19. Sprague SM, Belozeroff V, Danese MD, Martin LP, Olgaard K. Abnormal bone and mineral metabolism in kidney transplant patients – a review. Am J Nephrol 2008; 28: 246-253.
20. Muirhead N, Zaltman JS, Gill JS, et al. Hypercalcemia in renal transplant patients: prevalence and management in Canadian transplant practice. Clin Transplant 2014; 28: 161-165.
21. Amin T, Coates PT, Barbara J, Hakendorf P, Karim N. Prevalence of hypercalcaemia in a renal transplant population: a single centre study. Int J Nephrol 2016; 2016: 7126290.
22. Bonthuis M, Busutti M, van Stralen KJ, et al. Mineral metabolism in European children living with a renal transplant: a European society for paediatric nephrology/european renal association European dialysis and transplant association registry study. Clin J Am Soc Nephrol 2015; 10: 767-775.
23. Wong EK, Husain A, Sayer JA. Sarcoidosis presenting with hypercalcaemia following withdrawal of long-term immunosuppression in renal transplantation. Oxf Med Case Rep 2014; 2014: 86-88.
24. Evenepoel P, van Den Bergh B, Naesens M, et al. Calcium metabolism in the early posttransplantation period. Clin J Am Soc Nephrol 2009; 4: 665-72.
25. Lou I, Schneider DF, Leverson G, Foley D, Sippel R, Chen H. Parathyroidectomy is underused in patients with tertiary hyperparathyreoidism after renal transplantation. Surgery 2016; 159: 172.
26. Schwarz A, Rustien G, Merkel S, Radermacher J, Haller H. Decreased renal transplant function after parathyroidectomy. Nephrol Dial Transplant 2007; 22: 584-591.
27. Evenepoel P, Claes K, Kuypers DR, Debruyne F, Vanrenterghem Y. Parathyroidectomy after successful kidney transplantation: a single Centre study. Nephrol Dial Transplant 2007; 22: 1730-1737.
28. Molinari P, Alfieri CM, Mattinzoli D, et al. Bone and mineral disorder in renal transplant patients: overview of pathology, clinical, and therapeutic aspects. Front Med 2022; 9: 821884.
29. Schechter A, Gafter-Gvili A, Shepshelovich D, et al. Post renal transplantation anemia: causes, severity and their association with graft and patient survival. BMC Nephrol 2019; 20: 51.
30. Falkiewicz K, Nahaczewska W, Boratynska M, et al. Tacrolimus decreases tubular phosphate wasting in renal allograft recipient. Transplant Proc 2003; 35: 2213-2215.