

Serum Phosphate Measured at 6 and 12 Months After Successful Kidney Transplant Is Independently Associated With Subsequent Graft Loss

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Abstract

Objectives: Serum phosphate concentrations have been shown to predict graft loss in prevalent, but not incident, kidney transplant populations. The reasons for this are unknown. We investigated whether serum phosphate at 6 or 12 months posttransplant was associated with graft loss in the same cohort.

Materials and Methods: Data were collected for 325 patients transplanted and followed up at a single center (1996-2004). The association between serum phosphate at 6 and 12 months posttransplant and graft failure was analyzed.

Results: Univariable associations with death-censored graft failure were seen for serum phosphate at 6 and 12 months (hazard ratio [HR] 1.33; 95% confidence interval [CI] 1.20-1.48; $P < .001$, and HR 1.40; CI 1.27-1.54; $P < .001$). On bivariable analysis (phosphate at 6 vs 12 mo), a significant association remained for both variables and increased graft failure rate (HR 1.19; CI 1.07-1.34; $P = .002$, and HR 1.37; CI 1.21-1.55; $P < .001$). These associations persisted in multivariable models (HR 1.27; CI 1.07-1.51; $P = .007$, and HR 1.34; CI 1.14-1.57; $P < .001$ for phosphate at 6 and 12 mo).

Conclusions: Serum phosphate at 6 and 12 months posttransplant is an independent predictor of graft

loss. Any future trial designed to investigate the benefits of phosphate lowering should consider recruiting patients as early as 6 months posttransplant.

Key words: Hyperphosphatemia, Hypophosphatemia, Phosphatonin

Introduction

The outcomes of renal transplant have improved considerably since the early days of transplant.¹⁻⁵ Newer immunosuppression has improved graft survival rates with a reduction in acute rejection episodes. One-year graft survival rates have increased from 45% to 55% in the 1970s to 85% to 95% in the 1990s. Although the short-term success of kidney transplant has improved considerably, long-term success still needs improvement with 60% of deceased-donor transplant recipients developing graft failure within 10 years.

Serum phosphate has been shown to predict a decline in renal function in patients with native chronic kidney disease.^{6, 7} We⁸ and others⁹ have demonstrated that serum phosphate predicts graft survival posttransplant. This finding, however, has not been universal, with other investigators demonstrating no association.^{10, 11} These negative studies were performed in incident transplant patients (newly transplanted), whereas positive studies have been in prevalent cohorts (patients with transplants of different ages). Right after transplant, there is a high prevalence of hypophosphatemia as a consequence of hyperphosphatemia and renal phosphate wasting, with this effect normally resolving within the first year after transplant.¹² We therefore hypothesized that serum phosphate measured within 6 months of transplant might not

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be associated with graft loss, whereas late serum phosphate would.

Materials and Methods

Between January 1996 and December 2006, 325 patients at the renal unit at the Queen Elizabeth Hospital, Birmingham, United Kingdom, underwent renal transplant at that hospital. Before the study, the protocol was approved by our local institutional ethics committee, and conforms with the ethical guidelines of the 1975 Helsinki Declaration. Written, informed consent was obtained from all patients. All kidneys underwent static cold storage. Subsequent immunosuppression was based on calcineurin inhibitors in all patients with the majority also receiving azathioprine (Table 1). No patients received antithymocyte globulin (ATG) induction. Glomerular filtration rate (GFR) was estimated (eGFR) by the 4-variable Modification of Diet in Renal Disease formula with serum creatinine recalibrated to be traceable to an isotope derived mass spectroscopy method.¹³ Albuminuria was measured using a spot albumin: creatinine ratio.

The primary outcome of interest was death-censored graft failure, defined as *the requirement for dialysis reinstitution or retransplant*. These data were retrieved from the transplant database and cross-checked with data from the UK Transplant Registry held by the National Health Service Blood and Transplant. Secondary analyses were conducted to investigate the effect of serum phosphate on overall graft failure (including death with graft function).

Statistical Analyses

Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 16.0, IBM Corporation, Armonk, New York, USA). Data distribution was tested using the Kolmogorov-Smirnov test. Nonnormally distributed data were log-transformed before analysis. Numeric data were compared by the *t* test, and categorical data were analyzed by the chi-square test. Data are shown as means $\pm$ standard deviation or as median (interquartile range) as appropriate.

Initially, the relation between serum phosphate at 6 and 12 months after transplant and death-censored graft failure was investigated in separate univariable Cox proportional hazard models. The comparative effect of serum phosphate at 6 and 12 months was

then compared in a bivariable model. Bivariable analysis is a simple (2 variable) variant of multivariable analysis (where the relations between multiple variables are examined simultaneously). Finally, both metrics were examined in multivariable analyses adjusted for other relevant confounders at the same time points. These included donor and recipient age and sex, regraft, total human leukocyte antigen mismatch, systolic blood pressure, eGFR, serum calcium, and log-transformed parathyroid hormone and urinary albumin: creatinine ratio were measured at 6 and 12 months. Colinearity between explanatory variables was assessed by examining the variance inflation factor, with a variance inflation factor greater than 5 suggesting relevant colinearity. Kaplan-Meier analysis was used as a graphic display of the time to event analysis. The log-rank test was used to determine if there were significant differences between survival curves. A type 1 error rate below 5% ($P < .05$) was considered statistically significant.

Results

Three hundred twenty-five patients at the renal unit at the Queen Elizabeth Hospital Birmingham were transplanted from January 1996 to December 2004. Of these, 28 (9%) did not have a functioning graft after 12 months, leaving 297 available (91%) for analysis. Recipient and donor demographics, primary immunosuppression, human leukocyte antigen (HLA) mismatches, follow-up, and outcome data are shown in Table 1. Median length of follow-up, after the first 12 months, was 67 months (range, 0.06 to 135 months). During this period 11 patients (4%) died, and 22 (8%) lost their graft.

Biochemical characteristics of the study group at 6 and 12 months are shown in Table 2. Mean eGFR increased from 46 ± 18 mL/min/1.73 m² at 6 months to 48 ± 19 mL/min/1.73 m² at 12 months ($P = .01$). Mean serum phosphate concentrations increased from 1.02 ± 0.27 mmol/L at 6 months to 1.06 ± 0.27 mmol/L at 12 months ($P = .008$). Phosphate within the target range (as defined by the 2009 Kidney Disease: Improving Global Outcomes Initiative (KDIGO) guidelines¹⁴) (0.8 to 1.2 mmol/L) was present in 164 patients (55%) at 6 months, and 175 patients (59%) at 12 months (χ^2 : $P < .001$); hyperphosphatemia was present in 68 patients (23%) at 6 months, and 80 patients (27%) at 12 months

Table 1. Patient demographics.

Recipient age (y)	44 ± 14
Recipient sex male (%)	179 (60)
Graft number (%)	
1	261 (88)
2	31 (10)
3	4 (1)
4	1 (0.3)
Donor age (y)	45 ± 14
Donor sex male (%)	183 (57)
Donor type	
Deceased donor	240 (81)
Deceased donor + liver	1 (0.3)
Donation after cardiac death	0 (0)
Living related	42 (14)
Living unrelated	14 (5)
HLA mismatches	2.19 ± 1.32
Primary immunosuppression	
Prednisolone	297 (100)
Tacrolimus	15 (5)
Cyclosporine	282 (95)
Azathioprine	289 (97)
Mycophenolic acid	8 (2)
Induction with Anti-CD25 antibody	70 (24)

Table 2. Biochemical parameters at 6 and 12 months posttransplant.

	6 months	12 months	P value
Urea (mmol/L)	12.6 ± 47	12.3 ± 48	.9
Creatinine (μmol/L)	160 ± 85	157 ± 96	.4
eGFR (mL/min/1.73 m ²)	46 ± 18	48 ± 19	.01
Calcium (mmol/L)	2.49 ± 0.18	2.47 ± 0.17	.03
Phosphate (mmol/L)	1.02 ± 0.27	1.06 ± 0.27	.008
Alkaline phosphatase (IU/L)	339 ± 193	305 ± 185	< .001
Albumin (g/L)	41.5 ± 3.6	42.1 ± 3.6	.002
Parathyroid hormone (ng/mL)	91 (56-162)	94 (52-154)	.1
Phosphate ≥ 1.2 mmol/L (%)	70 (24)	81 (27)	< .001
Phosphate < 0.8 mmol/L (%)	62 (21)	39 (13)	< .001

Abbreviations: eGFR, estimated glomerular filtration rate

($P < .001$); hypophosphatemia in 64 patients (22%) at 6 months, and in 42 patients (14%) at 12 months ($P < .001$). Of those patients who were hyperphosphatemic at 6 months, 44 (65%) remained hyperphosphatemic at 12 months. The mean serum phosphate of the remaining 24 patients (35%) was 1.05 ± 0.08 mmol/L. None of these patients had hypophosphatemia with a serum phosphate of less than 0.8 mmol/L.

Table 3 shows the results for the death-censored graft failure analysis. Univariable analyses indicated that serum phosphate at 6 and 12 months was significantly associated with death-censored graft failure when examined individually (hazard ratio [HR] 1.33; 95% confidence interval [CI] 1.20-1.48; $P < .001$ and HR 1.40; 95% CI 1.27-1.54; $P < .001$). Donor age, recipient age, HLA mismatch and urinary albumin:creatinine ratio were also associated with death-censored graft failure in univariable analysis

Table 3. Adjusted and unadjusted proportional hazards regression for censored and uncensored graft loss per 0.1 mmol/L serum phosphate concentration.

	Graft loss (death censored)		Graft loss (not censored)	
	Hazard ratio	P value	Hazard ratio	P value
Univariable analysis				
Phosphate at 6 months	1.33 (1.20-1.48)	< .001	1.29 (1.17-1.41)	< .001
Phosphate at 12 months	1.40 (1.27-1.54)	< .001	1.33 (1.22-1.46)	< .001
Bivariable analysis				
Phosphate at 6 months	1.19 (1.07-1.34)	.002	1.17 (1.01-1.30)	.002
Phosphate at 12 months	1.37 (1.21-1.55)	< .001	1.30 (1.16-1.46)	< .001
Multivariable analysis*				
Phosphate at 6 months	1.27 (1.07-1.51)	.007	1.22 (1.06-1.40)	.006
Phosphate at 12 months	1.34 (1.14-1.57)	< .001	1.30 (1.13-1.50)	< .001

*Adjusted for donor and recipient age and sex, HLA mismatch, estimated GFR, serum calcium, systolic blood pressure, and log-transformed parathyroid hormone and urinary albumin:creatinine ratio.

($P < .01$ for all). When serum phosphate at 6 and 12 months after transplant were examined together in bivariable analysis, a significant association remained for both variables and increased graft failure rate (HR 1.19; 95% CI 1.07-1.34; $P = .002$, and HR 1.37; 95% CI 1.21-1.55; $P < .001$). There was no relevant colinearity between variables with a variance inflation factor of 1.483.

Figure 1 highlights the important observation that a serum phosphate higher than the recommended 1.2 mmol/L at 6 months and 12 months is associated with increased death-censored graft failure compared with a serum phosphate concentration below this level. Similar results for graft failure were obtained when phosphate ranges according to the KDIGO ranges of less than 0.8 mmol/L, 0.8-1.2 mmol/L, and greater than 1.2 mmol/L were compared (log rank 0.014 and 0.015 for serum phosphate at 6 and 12 months).

Finally, serum phosphate at 6 and 12 months were examined in a multivariable model adjusted for the following covariates: donor and recipient age and sex, regraft, total human leukocyte antigen mismatch, systolic blood pressure, eGFR, serum calcium, and log-transformed parathyroid hormone and urinary albumin: creatinine ratio all measured at 6 and 12 months. Serum phosphate at 6 and 12 months remained significantly associated with increased graft failure (HR 1.27; 95% CI 1.07-1.51; $P = .007$, and HR 1.34; 95% CI 1.14-1.57; $P < .001$). Recipient age and HLA mismatch also were associated with increased graft failure on multivariable analysis ($P < .01$ for both).

The analysis was repeated for overall graft survival, with similar results as shown in Table 3.

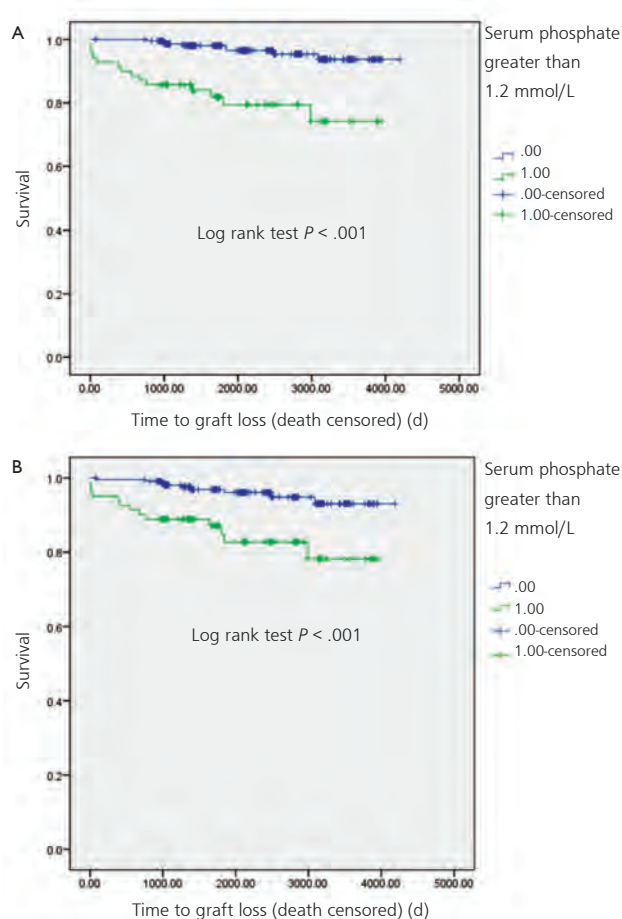


Figure 1. Kaplan-Meier survival analysis assessing death censored graft loss according to a serum phosphate level above or below 1.2 mmol/L at (A) 6 months and (B) 12 months after successful renal transplant.

Both serum phosphate at 6 and 12 months showed an association with graft failure rates on univariable, bivariable, and multivariable analyses. No association between serum phosphate and mortality was found.

Discussion

In this study, we have shown for the first time that serum phosphate at both 6 and 12 months after kidney transplant is associated with death-censored and overall graft survival in an incident recipient population. These associations persisted after adjustment for age, sex, blood pressure, proteinuria, donor type, and HLA mismatches. Patients with a serum phosphate concentration higher than the recommended 1.2 mmol/L had significantly poorer graft survival than those with lower values. These findings may have significant implications for future studies aimed at improving long-term graft survival.

Two other studies, with a similar number of incident transplant patients to our own, did not

demonstrate an association between serum phosphate and graft loss. Roodnat and associates¹¹ examined phosphate at only 2 months posttransplant in 407 patients. Hypophosphatemia and associated hypophosphatemia can persist for up to 12 months after transplant,¹² and therefore could have masked any relation between serum phosphate and graft survival at this early stage after transplant.¹⁰ Our findings are consistent with this, with the mean serum phosphate increasing from 6 months to 12 months, despite a rise in eGFR, as well as a decrease in the prevalence of hypophosphatemia. Egbuna and associates¹⁰ considered serum phosphate only as a categorical value of being low (< 0.8 mmol/L) or high (> 1.5 mmol/L) at several time points. Phosphate concentrations greater than 1.5 mmol/L were a rare occurrence, whereas the incidence of an abnormally high calcium-phosphate product was much higher. They found a strong association between calcium-phosphate product and graft survival. Thus, our starting hypothesis that serum phosphate at 6 months would not be associated with graft survival was incorrect, and that this is not the explanation for why the discussed studies were not found in this relation. This discrepancy is more likely to be related to other methodologic issues related to the timing of the analysis and the classification of phosphate status as discussed.

The mechanism underlying the association between serum phosphate and graft failure is unknown. A Western diet is typically rich in bioavailable phosphate, resulting in high renal phosphate load.¹⁵ A study of variable dietary phosphate content in uremic rat models in the 1980s provided interesting insights into potential mechanisms of kidney damage.¹⁶ Higher dietary phosphate was associated with greater urinary phosphate excretion and greater calcium and phosphate deposition in the renal tubules and interstitium of subtotal nephrectomized rats. Interstitial edema and fibrosis, together with tubular atrophy and dilatation, were present histologically. The nephrotoxicity of phosphate appeared to increase as renal functional mass decreased. It is hypothesized that high intracellular phosphate promotes precipitation of calcium-phosphate product within renal tubules, or that phosphate itself acts as a direct tubular toxin.¹⁷ Dietary restriction of phosphate reduces progression of kidney disease in animal models.¹⁸ In uremic rat models, the noncalcium-based

phosphate binder, sevelamer, reduced renal calcium content and appeared to protect against further deterioration in renal function.^{19, 20}

Serum phosphate also has been established as a predictor of cardiovascular mortality in the general population and in chronic kidney disease, including renal transplant and dialysis patients.^{8, 21-25} Strong evidence suggests that much of this cardiovascular risk can be attributed to the effects of phosphate on the vasculature and myocardium.¹⁵ Considering the strong relation between cardiovascular risk and rate of progression of renal disease, the influence of phosphate on the decline in renal function is another potentially important mechanism.

In our studied population, there were only 11 deaths during the follow-up, and this is probably the likeliest explanation why we were unable to show any relation between serum phosphate and mortality. Other studies in prevalent populations with high mortality rates have shown a significant association between serum phosphate and patient death.^{8, 21, 24}

Limitations

There are potential limitations to our study. We had only 2 measurements of serum phosphate (at 6 and 12 months), and thus, are unable to determine whether the association between serum phosphate and graft survival occurs before 6 months. We did not collect data on serum vitamin D or fibroblast growth factor-23 concentrations, nor on the subsequent use of phosphate binders, calcium supplements, or vitamin D analogues; these factors might have provided greater insight into potential causative mechanisms. Although prospective in nature, the study was observational and as such, was subject to potential residual confounding from missing variables. Survivor bias also has to be considered when enrolling only patients who had survived for more than 12 months, and this might explain the rising GFR with time. Despite this, it is known that event rates remain broadly stable beyond 6 months after transplant, even when patients are studied many years after transplant.^{26, 27}

Conclusions

We have shown serum phosphate to be an independent predictor of graft loss in incident kidney transplant recipients. Further work is required to determine the underlying mechanisms by which

phosphate influences graft loss. Randomized controlled trials are warranted to investigate the potential benefits of phosphate lowering as a target for intervention in transplant recipients. Such trials should start at 6 months after the transplant or earlier.

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