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ORIGINAL ARTICLE

Mineral adaptations following kidney transplantation

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SUMMARY

Klotho is predominantly expressed in the kidney and reported to have antioxidant and antifibrotic properties. Soluble Klotho (sKl), the circulating protein cleaved from membrane-bound Klotho, is reduced significantly with kidney disease and inversely associated with mortality. sKl has not been thoroughly evaluated prospectively after kidney transplantation. Incident kidney transplant recipients (KTRs) were prospectively evaluated pre-transplantation, 1, 12 and 52 weeks post-transplantation. Basic biochemistry, sKl and intact FGF23 were measured. Within-subject comparisons were evaluated using repeat-measure ANOVA or Friedman's analysis. Effects of immunosuppression and biochemical parameters on sKl and FGF-23 over time were analysed using mixed-effects modelling. Median serum creatinine (sCr) at 1 week was 116 (92–142) μmol/l, and at 52 weeks, all 29 KTRs had a functioning graft with median sCr of 111 (97–131) μmol/l. Compared with baseline, sKl was increased at 52 weeks following an initial decline at 1 week ($P < 0.005$ and $P < 0.01$, respectively), while FGF23 was considerably reduced at 52 weeks ($P < 0.001$). In a mixed-effects model, an increased sKl was not associated with reduction in immunosuppression or evaluated biochemical parameters. Modest increase in sKl is observed one-year postkidney transplantation with excellent early graft function suggesting factors beyond renal capacity may influence circulating sKl. FGF23 normalization was observed. Longer term evaluation in transplantation, specifically addressing the effects of immunosuppression, is required to understand the pathophysiology of the sKl/FGF23 axis and potential for modification.

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Background

Kidney transplantation remains the treatment of choice for patients with end-stage renal disease (ESRD), contributing to better survival and improvement in quality of life [1]. Kidney transplantation significantly improves, and often resolves, some complications of chronic kidney disease (CKD) such as anaemia and

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hyperkalaemia. However, biochemical parameters of mineral metabolism can remain impaired in kidney transplant recipients (KTRs) with persistent hyperparathyroidism, hypercalcaemia and hypophosphatemia observed as common problems [2].

Abnormalities of mineral metabolism are almost universal complications of CKD and are associated with bone disease, vascular calcification, accelerated atherosclerosis and excess cardiovascular morbidity. These associations are now better appreciated within the entity 'chronic kidney disease–mineral bone disorder' (CKD-MBD) [3]. While changes to serum phosphate levels are a hallmark of advanced CKD and ESRD, evaluation of the recently discovered fibroblast growth factor-23 (FGF23) and soluble Klotho (sKl) have provided insight into changes that occur much earlier in CKD. Markedly elevated FGF23 levels have been consistently reported with progressive decline in kidney function, and increased levels have also been linearly correlated with increased mortality across all stages of CKD, independent of traditional vascular risk factors [4–8]. Conversely, a reduction in sKl is observed with kidney dysfunction [9] and an inverse correlation between sKl levels with mortality has been reported [10].

Determining the impact of kidney transplantation on these newer biomarkers of CKD-MBD will further the understanding of this disorder, with the potential for identifying targets to improve outcomes in kidney transplantation. FGF23 decreases dramatically in the early post-transplantation period, and studies demonstrate further decline by 12 months to similar levels observed in CKD patients matched for estimated glomerular filtration rate (eGFR) [11–14]. There is a paucity of data, however, evaluating changes in sKl following kidney transplantation. One small prospective study reported no significant change in KTRs after 5 days [15], while three cross-sectional studies reported conflicting results in long-term KTRs [16–18].

The kidneys are considered a major source of circulating sKl [19,20], a protein thought to have renal and extra-renal effects beyond phosphate metabolism including ion channel signalling activity, antioxidative and antifibrotic properties [21]. However, a rise in sKl levels has not been convincingly shown in renal transplantation and, to our knowledge, there have been no prospective studies beyond the first week, evaluating sKl in incident KTRs. We hypothesized that an increase in sKl may occur following kidney transplantation and therefore evaluated sKl levels in incident KTRs over the first 12 months following transplantation.

Patients and methods

Study population

Cohorts from two multicentre, prospective studies involving incident KTRs, which had completed recruitment in Victoria, Australia, were studied for changes in sKl post-transplantation. Both studies evaluated identical time-points following kidney transplantation. A *post hoc* analysis was conducted using stored serum samples from incident KTRs, above the age of 18 years, recruited from various Victorian nephrology units involved in these two studies. Exclusion criteria included active cardiovascular disease, malignancy, chronic infection, inflammatory disease, psychological or medical illness precluding informed consent and lack of baseline sample for comparison. Studies were approved by local human research ethics committees and conducted in accordance with the Declaration of Helsinki.

Data collection, sample collections, and biochemical analysis

General demographic information, disease aetiology and pretransplantation history were collected for all study participants. Post-transplantation management and immunosuppression monitoring were strictly undertaken by individual treating units or physicians, according to hospital specific protocols without input from research personnel. Immunosuppression doses and drug levels at study time-points were documented.

Participants were sampled at baseline (pretransplantation), 1 week (1 w), 12 weeks (12 w) and 52 weeks (52 w) post-transplantation. Routine biochemistry, including haemoglobin (Hb), serum calcium (sCa), phosphate (sPi), albumin (Alb) and creatinine (sCr), were performed at all time-points through respective on-site pathology laboratories. eGFR was calculated using the CKD-EPI equation [22]. Additional blood samples were collected, processed and aliquoted for storage at −80 °C until batched analysis. Serum sKl concentrations were measured using the IBL soluble Klotho ELISA kit (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) according to the manufacturer's protocol. Based on duplicate measurements, the intra-assay and interassay CVs for this study were 3.6% and <5%, respectively. Intact FGF23 was assayed using the Kainos intact FGF23 assay (Kainos Laboratories, Tokyo, Japan) where intra-assay and interassay CVs were 4.1% and 7%, respectively. Intact parathyroid hormone

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(PTH) was evaluated at baseline and 52 w, while 25-hydroxy vitamin D levels were assessed at baseline only.

Statistical analysis

Continuous variables have been reported using mean and standard deviation (SD) or median and interquartile range (IQR) as appropriate, while categorical variables have been summarized using frequency and percentage. Within-subject changes in measured parameters over time were evaluated using Friedman test with Dunn's multiple comparisons for non-normally distributed values and repeated-measures ANOVA for normally distributed values.

Non-normally distributed variables were natural log-transformed (Ln) for mixed-effects model analyses. Mixed-effects models were used to assess the temporal associations between parameters. Firstly, the effect of improved renal function, variations in (Ln)sKl, (Ln) iFGF-23 and calcium on the change in phosphate levels was analysed. Subsequently separate analyses were undertaken to assess the effect of improved eGFR, alterations in serum phosphate and calcium, and changes in immunosuppression on either (Ln)sKl levels or (Ln) iFGF-23 across time-points. All mixed-effects model analyses were performed adjusting for age as an independent linear covariate and allowing for the random effect of management by different nephrology units. All statistical analyses were performed with SPSS STATISTICS Version 24.0 (IBM Corp., Armonk, NY, USA). Graphics were created with GRAPHPAD PRISM 6 for Macintosh (La Jolla, CA, USA). *P*-value of <0.05 was considered significant unless otherwise stated.

Results

Study patients and immunosuppression

Thirty-four incident KTRs were recruited. Four did not complete all study time-points. One patient was diagnosed with Epstein–Barr virus-associated post-transplant lymphoproliferative disorder necessitating withdrawal of immunosuppression at 10 months following kidney transplantation and hence was excluded from further analysis. A total of 29 KTRs were included for final study analysis. Seventeen (58.6%) were male. Median age [and interquartile range (IQR)] of participants was 49 (35–55) years. Demographic information and biochemical profile of study participants are displayed in Table 1. In the cohort evaluated, 89.6% ($n = 26$) of kidney transplants were from a living donor. Two of three deceased donor KTRs were complicated by delayed graft function (DGF). No living-donor kidney allograft had DGF. Median (IQR) sCr at baseline and 1 w post-transplantation was 638 (537–722) and 116 (92–142) μmol/l, respectively. At 52 w, all 29 KTRs had a functioning graft with a median (IQR) sCr of 111 (97–131) μmol/l.

Table 1. Baseline demographic of study participants ($n = 29$).

Clinical characteristics	Value*
Age, years	49 (35–55)
Gender (male), *n* (%)	17 (59%)
Body mass index (BMI), kg/m^2	25.5 ± 4.4
Kidney disease aetiology	
Glomerulonephritis	11 (38%)
Diabetic nephropathy	0
Vascular disease	2 (7%)
Polycystic kidney disease	6 (21%)
Reflux nephropathy/congenital disease	3 (10%)
Tubulointerstitial disease	2 (7%)
Other/unknown	5 (17%)
Kidney transplant type	
Living donor	26 (90%)
Deceased donor	3 (10%)
Dialysis modality (pretransplantation)	
Peritoneal dialysis	3
Home haemodialysis	3
Satellite haemodialysis	12
Pre-emptive transplant/no prior dialysis	11
Biochemical parameters	
Serum phosphate (sPi), mmol/l	1.78 ± 0.5
Serum calcium (sCa), mmol/l	2.40 ± 0.18
Serum albumin, g/l	36 ± 4
Serum creatinine (sCr), μmol/l	638 (537–722)
eGFR, ml/min/1.73 m^2	7.4 (6.5–8.7)
Parathyroid hormone, pmol/l	21.7 (13.2–33.7)
25-OH vitamin D, nmol/l	43 (35–53)
s-Klotho, pg/ml	307 (279–460)
Intact FGF-23, pg/ml	2060 (825–5075)

*Normally distributed variables presented as mean ± SD; non-normally distributed variables presented as median (IQR).

Immunosuppression effects

All KTRs received interleukin-2 (IL-2) antagonist induction therapy and traditional triple agent immunosuppression, including calcineurin inhibitor, antiproliferative agent and corticosteroid. Two different types of calcineurin inhibitors (CNI) were used, and twenty-five (86%) of KTRs treated with tacrolimus while four were treated with cyclosporine. KTRs were managed at respective treating units with site-specific immunosuppression protocols, although the overriding management

Table 2. Mean level of immunosuppression (n = 29).

Immunosuppression	1 week	12 weeks	52 weeks
Prednisolone (mean daily dose, mg)	27.9 ± 3.5	11.2 ± 3.6[a]	5 ± 1.8*[,a,b]
Mycophenolate (mean daily dose, mg)	2017 ± 211	1278 ± 585[a]	1000 ± 500[a]
Tacrolimus† (mean trough level, ng/ml)	7.2 (6.4–8.6)	7.65 (5.9–9.3)	5.3 (4.5–6.4)[a,c]
Cyclosporine† (median 2 h postdose level, ng/ml)	1315 (1100–1605)	737 (658–859)	459 (162–715)

Data presented as mean ± SD or Median (IQR). Friedman test with Dunn's multiple comparisons performed for non-normally distributed values and repeated-measures ANOVA performed for normally distributed values.

*All patients were on 5 mg prednisolone daily except two.

†25 (86%) study participants were treated with tacrolimus and 4 (14%) on cyclosporine.

[a]$P < 0.005$ compared with 1 week. [b]$P < 0.005$ compared with 12 weeks. [c]$P < 0.05$ compared with 12 weeks.

Table 3. Change in mineral metabolism parameters subsequent to kidney transplantation (n = 29).

Parameter	Baseline	1 week	12 weeks	52 weeks
s-Klotho, pg/ml	307 (279–460)	273 (246–343)[b]	352 (286–417)[c]	460 (311–525)[a,c]
i-FGF-23, pg/ml	2060 (825–5075)	144 (54–351)[a]	71 (46–122)[a]	64 (34–88)[a,d]
Serum phosphate (sPi), mmol/l	1.78 ± 0.5	0.81 ± 0.29[a]	0.88 ± 0.19[a]	0.92 ± 0.17[a]
Serum calcium (sCa), mmol/l	2.40 ± 0.18	2.37 ± 0.20	2.46 ± 0.13	2.44 ± 0.13
Serum albumin, g/l	36 ± 4	31 ± 3[a]	38 ± 3c	38 ± 4[b,c]
Serum creatinine (sCr), µmol/l	638 (537–722)	113 (92–142)[a]	112 (99–130)[a]	111 (97–131)[a]
eGFR, ml/min/1.73 m^2	7.4 (6.5–8.7)	63.2 (46.5–87.4)[a]	61.2 (51.7–71.9)[a]	60.4 (50.5–71.6)[a]
intact PTH, pmol/l	21.7 (13.2–33.7)	n/a	n/a	7.2 (3.9–11.3)[a]

Data presented as mean ± SD or Median (IQR). Friedman test with Dunn's multiple comparisons performed for non-normally distributed values and repeated-measures ANOVA performed for normally distributed values.

[a]$P < 0.005$ compared with baseline. [b]$P \leq 0.01$ compared with baseline. [c]$P < 0.005$ compared with 1 week. [d]$P < 0.05$ compared with 1 week.

principles were comparable with similar pharmacological choices, drug availability and reduction in immunosuppression over the first 12 months. Mycophenolate levels (area under the curve) were not routinely performed at all institutions and thus not recorded for the purposes of this study. Average prednisolone dose, mycophenolate dose and CNI drug levels have been tabulated in Table 2. Prednisolone doses decreased over the 12 months following transplantation, while mycophenolate doses were reduced at 12 w compared to initial doses with no significant reduction between 12 and 52 w. Meanwhile, CNI levels were lower at 52 w compared to 1 and 12 w with no difference between 1 and 12 w.

Twelve episodes of rejection in eleven KTRs were recorded in this cohort over 52 w. Four KTRs experienced allograft rejection within the first month treated successfully with increased immunosuppression. No further episodes were observed within the remaining 52 w in this early rejection group although two subsequently were diagnosed with cytomegalovirus (CMV) viremia. Subclinical cellular rejection was identified on routine protocol biopsies in six other KTRs – four at 12 w and two at 52 w. These episodes were treated with increased overall immunosuppression and close monitoring. In another recipient, acute cellular rejection was diagnosed at 6 months post-transplantation and treated with increased corticosteroid therapy. Antibody-mediated rejection was diagnosed 3 months later, necessitating further immunosuppression with increased antiproliferative dosage in combination with plasma exchange therapy. This recipient maintained stable renal allograft function (sCr 97 µmol/l) at 52 w despite these rejection episodes.

Over the 52 w period, eight (27.6%) KTRs developed complications of over-immunosuppression with one case of nonspecific marrow suppression, one case of parvovirus infection, four cases of BK viremia and two cases of CMV viremia (already mentioned above). All

affected KTRs had immunosuppression reduced either with reduction in mycophenolate or change in CNI from tacrolimus to cyclosporine.

Mineral parameters after transplantation

Changes to mineral parameters following kidney transplantation have been tabulated in Table 3. At 1 w post-transplant, median (IQR) eGFR and sCr were 63.2 (46.5–87.4) ml/min/1.73 m^2 and 113 (92–142) μmol/l ($P < 0.001$ compared with baseline). Renal function remained stable with similar eGFR and sCr levels at 52 w [60.4 (50.5–71.6) ml/min/1.73 m^2 and 111 (97–131) μmol/l, Fig. 1].

Baseline sCa levels were within the normal range and no significant change over time was detected (Fig. 2a). Mean sPi was mildly elevated at baseline (1.78 ± 0.5 mmol/l), with normalization by 1 w ($P < 0.0001$ compared with baseline), sustained at both 12 and 52 w (Fig. 2b).

Serum sKl levels in KTRs across evaluated time-points are depicted in Fig. 3a. A significant reduction in sKl is demonstrated at 1 w following kidney transplantation compared to baseline [273 (246–343) vs. 307 (279–460) pg/ml, $P = 0.0068$] prior to a gradual increase in levels over the subsequent 11 months with levels at 52 w significantly higher than baseline [460 (311–525) vs. 307 (279–460) pg/ml, $P = 0.0015$]. Percentage change over time from baseline is shown in Fig. 3b.

Kidney transplant recipients treated with cyclosporine demonstrated lower sKl levels [311 (273–315) vs. 486 (362–561) pg/ml, $P = 0.016$] and a trend towards a smaller percentage change in sKl levels (+6% vs. +28%, $P = 0.08$) at 52 w compared to KTRs treated with tacrolimus (Fig. 4a). No other discrepancies were noted between patients treated with cyclosporine or tacrolimus.

No differences were noted in sKl levels in KTRs who exhibited evidence of any kidney allograft rejection

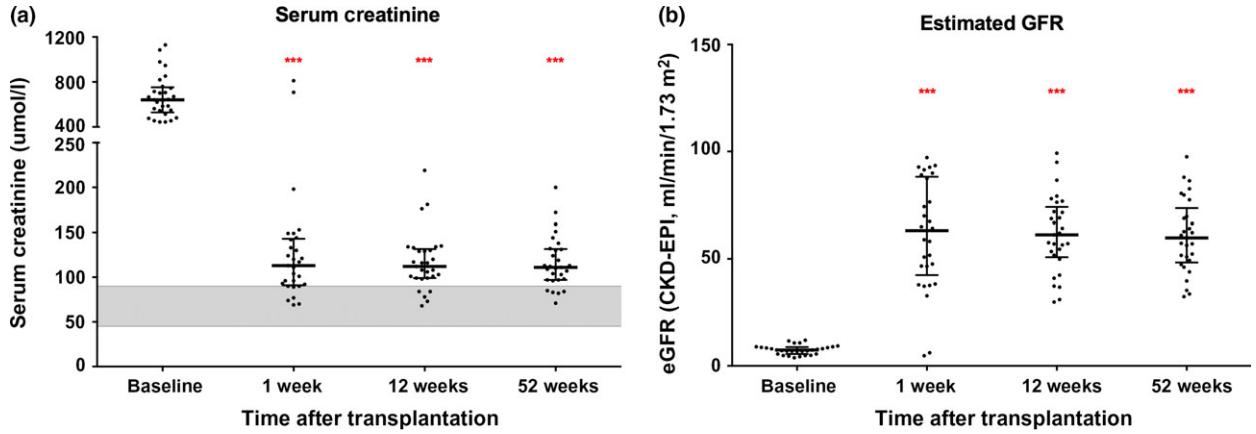

Figure 1 Renal function improves with kidney transplantation characterized by (a) a reduction in serum creatinine and (b) an increase in eGFR. ***$P < 0.001$ compared to baseline

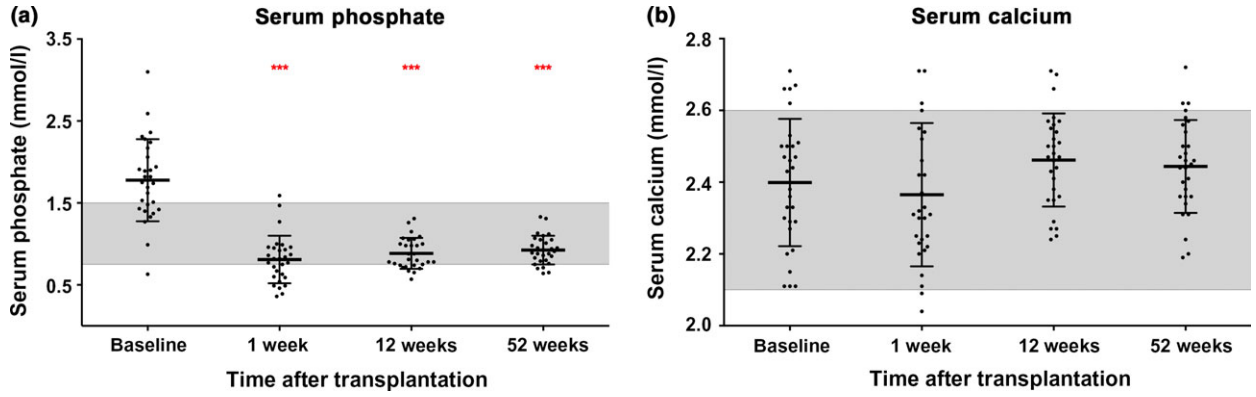

Figure 2 (a) Reduction in serum phosphate occurs while (b) no change is seen in serum calcium levels following kidney transplantation. ***$P < 0.001$ compared to baseline.

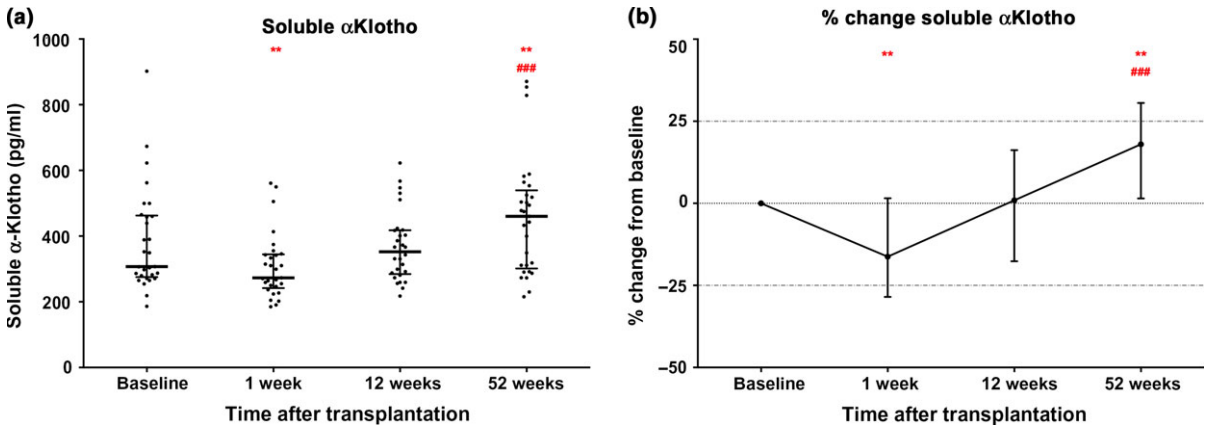

Figure 3 (a) Soluble Klotho (sKl) levels decrease at 1 week before a rise at 52 weeks. (b) Percentage change [median, interquartile range (IQR)] of sKl levels from baseline plotted. $^{**}P < 0.01$ compared to baseline, $^{###}P < 0.001$ compared to 1 week.

compared to those who did not (Fig. 4b). Similarly, there was no detectable difference in sKl levels in patients who developed complications of over-immunosuppression compared to the rest of the cohort at 12 and 52 w (Fig. 4c).

Significantly elevated baseline iFGF23 levels, 2060 (825–5075) pg/ml, showed a dramatic reduction at 1 w, 144 (54–351) pg/ml ($P < 0.0001$). These levels continued to decline in a more gradual fashion thereafter where median iFGF23 levels at 52 w were 64 (34–88) pg/ml ($P < 0.0001$ vs. baseline, $P = 0.014$ vs. 1 w, $P = 0.76$ vs. 12 w, Fig. 5). However, at 52 w more than 30% of the cohort ($n = 10$) continued to have iFGF23 levels above 70 pg/ml and more than 10% ($n = 4$) still had levels over 100 pg/ml.

Associations with change in Klotho

Utilizing a mixed-effects model allowing for random effects as specified, changes in eGFR, sCa and (Ln)iFGF-23 were significantly associated with the change seen in sPi over the four time-points evaluated. A change of −0.0034 ml/min/1.73 m^2 in eGFR ($P = 0.036$) and −0.45 mmol/l sCa ($P = 0.011$) while +0.08 in (Ln) iFGF23 ($P = 0.001$) was noted per unit change in sPi over time. Age and (Ln)sKl did not contribute to changes in sPi.

Improvement in eGFR and sCa was significantly associated with changes in (Ln)iFGF23. A change of −0.014 ml/min/1.73 m^2 in eGFR ($P = 0.011$) and +2.18 mmol/l sCa ($P = 0.002$) was noted per unit change in (Ln)iFGF23 over time. Neither normalization of sPi nor changes in (Ln)sKl were associated with change in (Ln)iFGF-23.

Soluble Klotho levels are reported to be associated with age and renal function; however, mixed-effects modelling showed increases in sKl over the 12-month post-transplant course were not associated with any of the following fixed variables; renal function, (Ln)iFGF-23, sPi, sCa, prednisolone dose, mycophenolate dose or CNI type after adjusting for age. Repeating the analysis evaluating only eGFR, (Ln)iFGF-23, sPi, sCa and age did not show any impact on (Ln)sKl over 12 months either.

Discussion

To our knowledge, this is the first study to prospectively evaluate sKl in KTRs over the first year postkidney transplantation. We report that sKl levels demonstrated an initial reduction within the acute post-operative phase followed by a gradual increase with the highest levels exhibited at 52 w post-transplant compared with baseline pretransplant levels. This finding supports our initial hypothesis. No significant association was noted between reduction in immunosuppression, improvement in renal function and change in iFGF-23 with the increase detected in sKl.

As hypothesized, sKl levels increased following kidney transplantation, although interestingly this was only observed at 52 w. While two KTRs had DGF, the majority (>85%) of the study cohort achieved acceptable renal allograft function at 1 w, where sCr was <150 μmol/l. Furthermore, renal function remained stable for the duration of the study between 1 and 52 w. As kidneys are considered a major source of circulating Klotho [20] and correlations between Klotho (both membrane and soluble levels) with eGFR have

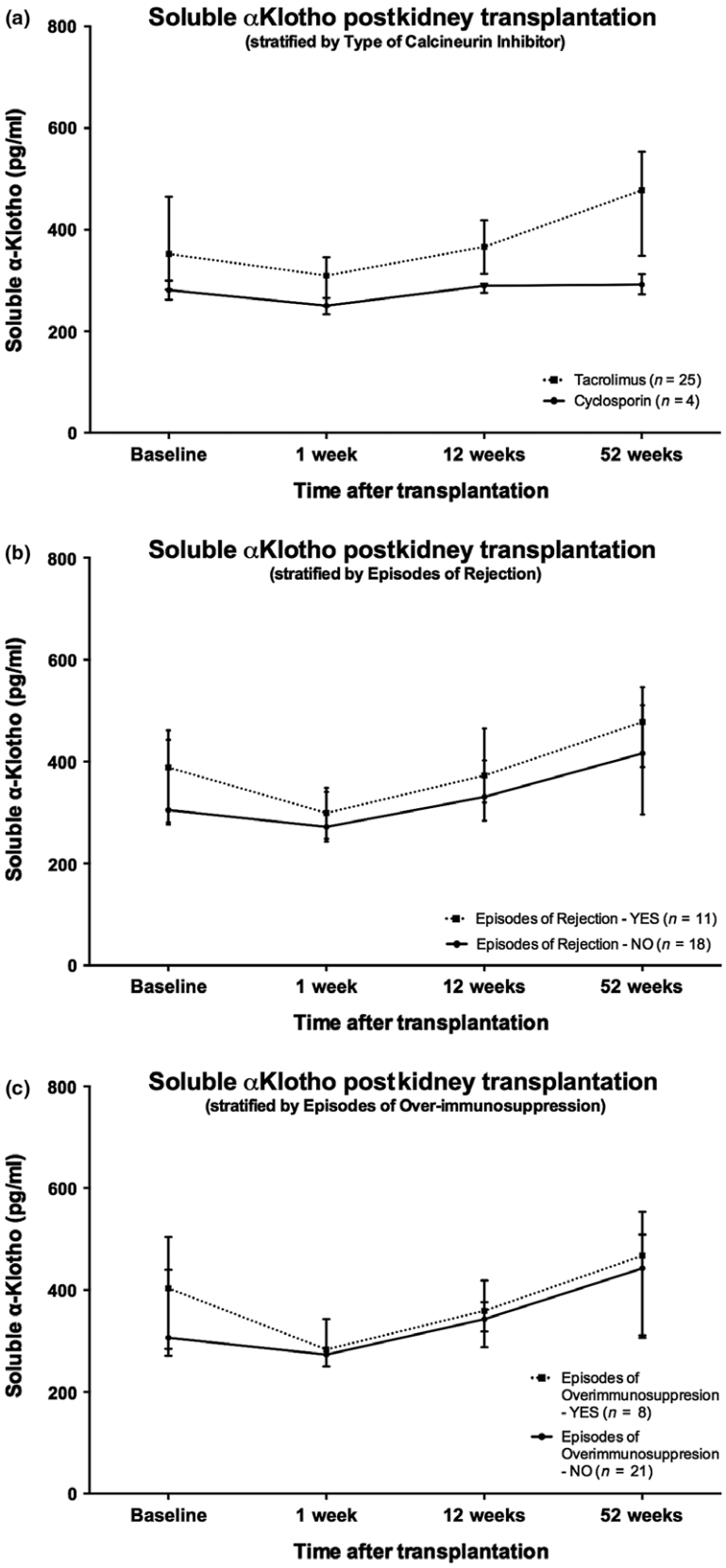

Figure 4 Soluble Klotho (sKl) levels over the study period were stratified according to (a) calcineurin inhibitor type – those on tacrolimus showed a trend to higher levels at 52 weeks compared to those on cyclosporine, (b) episodes of rejection and (c) episodes of over-immunosuppression. The latter two events did not seem to affect overall sKl levels.

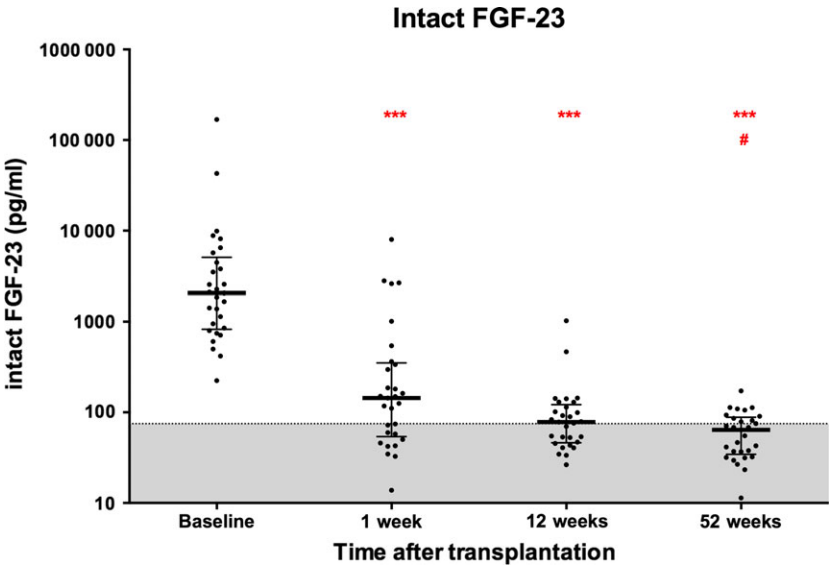

Figure 5 A substantial reduction in iFGF-23 levels was observed in the acute post-transplantation period. ***$P < 0.001$ compared to baseline, $^{\#}P < 0.05$ compared to 1 week.

been reported [23], an increase in sKl levels following renal transplantation was not unexpected. However, in the context of stable kidney function achieved by 1 w, this increase was anticipated earlier, perhaps by 12 w. Not only was this not observed in our study but also ~15% decrease in sKl was noted in the acute postoperative period, which suggests other mechanisms involved in the regulation of circulating sKl levels.

The initial decline in sKl levels observed within the acute postoperative period is likely multifactorial. Firstly, there may be a dilutional effect as KTRs are commonly administered a significant volume of fluid during this period. Secondly, many acute phase proteins alter significantly in the first 24–48 h postmajor surgery as a response to trauma and tissue injury [24] and may putatively impact cleavage and production of sKl. Thirdly, although Hu *et al.* [20] have recently demonstrated in animal models the fundamental role of kidneys in Klotho metabolism, both as a source of Klotho and a site of regulation determining cleavage and circulation of sKl, there are many facets of this pathway and regulatory process that are not fully understood including the time course in which it occurs in humans, as well as what and how other factors, such as physiological stress or pharmacological burden, may influence circulating sKl levels.

Furthermore, kidney allografts particularly renal tubules are subject to a period of rapid, intense physiological changes in the early post-transplantation period contributing to potential isosthenuria, tubular dysfunction [25] and abnormal urinary rhythm [26], albeit reversible in the majority of cases. As the predominant source of sKl [20], this transient tubular disruption may contribute to the decline observed in circulating sKl levels at 1 w. Finally, the first week following renal transplantation is a period of heavy immunosuppression with high-dose corticosteroid therapy immediately following induction therapy, in this case IL-2 inhibition. Immunosuppression may potentially contribute to lower sKl levels at 1 w postkidney transplantation, and certainly plausible links have been reported between immunosuppressive agents and lower Klotho expression in experimental models. However, this hypothesis requires further exploration of causal mechanisms.

While no statistically significant association was found between reduction in immunosuppression and increment in sKl in this small study, a trend for lower sKl levels in those treated with cyclosporine was notable. The unequal distribution of KTRs treated with the two different CNIs is a limitation to interpretation of this finding although animal cyclosporine nephropathy models [27–29] support the plausibility of lower Klotho expression while cyclosporine use in experimental solid organ transplant models has also been shown to reduce ADAM17 expression [30], a Klotho cleavage enzyme [31]. Taken together, the potential causal relationships and mechanisms between types and intensity of immunosuppression with Klotho expression and metabolism may warrant further investigation.

FGF23 levels declined postrenal transplantation, as reported previously [11–14], although at 52 w a proportion of these patients still had levels >70 pg/ml. A global iFGF23 reference range has not been adopted in view of the various commercial assays available and lack of direct comparison between them [32,33]. However, normal healthy volunteer mean (±SD) iFGF23 levels using

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the same assay used here have been reported at 42 ± 9.9 pg/ml [32], suggesting levels above 65–70 pg/ml lie beyond the 2SD limit.

There is currently no available data on Klotho expression or regulation in experimental models of kidney transplantation to aid interpretation of our study findings. There are a total of four published studies in KTRs evaluating sKl levels (Table 4) [15–18]. One small study of 10 KTRs documented a nonsignificant trend to lower sKl levels in the first 5 days following surgery [15]. The other three cross-sectional studies report conflicting results in KTRs evaluated at a median time of 3–18 years following renal transplantation when compared with age-matched healthy volunteers. One study of 84 KTRs at a median of 3-year follow-up reported lower sKl levels [16] while another study of 39 KTRs, with a median time since transplantation of 18 years, observed non-significant lower sKl levels [17], when compared to healthy volunteers. This is in direct contrast to 117 KTRs, assessed at a median time of 5 years following transplantation, who were observed to have higher sKl levels than healthy volunteers [18]. Of note, the latter study was the only report using a different sKl assay in the context of known analytical differences in commercially available assays [34] that have not yet been subjected to international standardization. Thus, the latter report of higher sKl in KTRs is inconsistent with other reports, and may reflect differences in assay performance.

Our study is important and adds to the understanding of mineral metabolism in CKD and kidney transplantation. The modest increment in KTRs when followed prospectively after kidney transplantation strengthens the concept of the kidney as a source of circulating Klotho. Concurrent evaluation of renal function and other biochemical parameters allowed for an assessment of the possible relationship between these variables and sKl. Despite early allograft function and significant flux in serum phosphate levels in this cohort of predominantly living-donor KTRs, a rise in sKl was only evident by 52 w in our study. Our findings are consistent with the short-term study involving KTRs assessed in the first 5 days post-transplant [15] and support the plausible notion of lower sKl in KTRs in the longer term when compared to age-matched healthy controls seen in other studies [16,17]. Moreover, our study suggests that renal function is not the only determinant of circulating sKl levels.

Limitations of our study include an absence of an age-matched healthy volunteer control group followed at the same time-points assessing the same parameters and the lack of contemporaneous evaluation of other specific mineral metabolism markers, such as 1,25 dihydroxycholecalciferol, and urinary samples for tubular function assessment or urinary sKl. The KTRs assessed in this study however remained their own controls in a repeat-measure statistical analysis and provide justified comparison. Of note, a separate cohort of healthy volunteers (of similar age) has been shown to have minimal change in sKl levels over a 52 w period (S.J. Tan, manuscript under review elsewhere). PTH values at all time-points would have strengthened the

Table 4. Summary of key findings in published studies evaluating soluble Klotho (sKl) in kidney transplant recipients (KTRs).

Study	Year	Type	Sample size, *n*	Comparator group	Assay used	Median follow-up (years)	Key finding
Akimoto *et al.* [15]	2013	Short-term Prospective	10	Kidney donors	IBL	n/a	No significant change over 5 days of follow-up
Malyszko *et al.* [16]	2014	Cross-sectional	84	Healthy Volunteers	IBL	3.1	Lower sKl levels in KTRs compared to control group
Leone *et al.* [18]	2014	Cross-sectional	117	Healthy Volunteers	Cusabio	5.2	Higher sKl levels in KTRs compared to control group
Bleskestad *et al.* [17]	2015	Cross-sectional	39	Healthy Volunteers; eGFR-matched controls	IBL	18.3	Lower sKl in KTRs compared to healthy volunteers and to lesser extent eGFR-matched controls

multivariable mixed-effect analysis although it is likely sCa levels performed as a surrogate, given its significant associations with changes in sPi and iFGF-23 despite lack of change in sCa over time. Lastly, urinary samples, urinary phosphate (and biochemical) measurements and Klotho levels would have (i) aided interpretation of sKl levels offering insight into degradation and metabolism of sKl and (ii) permitted a more intense interrogation of tubular function during the study.

Conclusion

In summary, we conclude that sKl levels increase following kidney transplantation although changes are modest compared to more significant improvements in renal function and iFGF-23 levels. Furthermore, sKl increase was only observed by 1 year post-transplantation, in contrast to the early changes in iFGF-23 levels and renal function in this cohort of predominantly living kidney allograft recipients. Taking into consideration the positive associations reported between sKl with survival, longer term studies dedicated to assessing cardiovascular and mortality outcomes as well as other CKD-MBD parameters such as bone health should be undertaken to assess the temporal relationship with sKl in renal transplantation.

Authorship

SJT: designed the *post hoc* study, conducted the assays, undertook the analysis and prepared the manuscript. AC, DL, VO and KMD: were primary drivers of the clinical studies. FLI, MAR and PDH: contributed intellectually to the critique and final draft of the manuscript. TDH, KMD and NDT: were involved in study design, assisted with analysis and manuscript preparation. All authors have critically revised and approved the manuscript.

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Conflict of interest

SJT has received speaking honoraria from Shire. NDT has received consultancy fees, honoraria and research funding from Amgen and Shire Pharmaceuticals.

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