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EFFECT OF CALCINEURIN INHIBITORS ON IONIC STATUS EARLY AFTER HSCT

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ABSTRACT

The calcineurin inhibitors (CNI) cyclosporine and tacrolimus are widely used as immunosuppressive agents in recipients of hematopoietic stem cell transplantation. Since limited data is available from India on this subject, the objective of this study was to retrospectively evaluate the impact of CNIs on the post-transplant level of serum magnesium, potassium and creatinine and compare the toxicity profile of the two commonly used CNIs cyclosporine and tacrolimus. We observed that 20/31 (64.5%), 15/31 (48.3%) and 8/31 (25.8%) patients had low magnesium, potassium and high creatinine respectively. At a 5% level of significance, serum magnesium showed a significant decline over a three week period in patients on cyclosporine (p value:0.017). The dosage of cyclosporine also correlated significantly with the maximum decrease in serum magnesium in the third week post-transplant (r: -0.620, p value: 0.014). We concluded that ionic imbalance is an important side-effect of CNIs and regular estimation of electrolytes is imperative for early detection of abnormal levels. Periodic evaluation of magnesium as an integral part of the diagnostic protocol in the immediate post transplant period particularly assumes greater significance. Appropriate correction of electrolyte imbalance would help prevent multiple co-morbid conditions.

KEYWORDS: Calcineurin inhibitors, haematopoetic stem cell transplant, magnesium, potassium, creatinine.

INTRODUCTION

Each year, approximately 50,000 patients worldwide undergo hematopoietic stem cell transplantation (HSCT). This procedure, which is used to treat a wide range of malignant and nonmalignant diseases, involves either a myeloablative or reduced-intensity conditioning regimen; the use of alternative allogeneic donor cells and immunomodulatory agents to prevent graft-versus-host disease (GVHD).

The introduction of immunosuppressive agents, the calcineurin inhibitors (CNI) cyclosporine in the late 1970s and tacrolimus in 1984, revolutionized transplantation medicine.[1,2,3,4] Most of the transplant recipients are discharged post-transplantation with a CNI-based regimen[5]. The immunosuppressive properties of cyclosporine and tacrolimus result from inhibition of calcineurin, a calcium- and calmodulin-dependent phosphatase (protein phosphatase 3[PPP3C]). Intracellularly, these molecules bind to cyclophylin and FKBP12 for cyclosporine and tacrolimus, respectively.[6,7,8,9] The competitive binding of cyclosporine-cyclophylin and tacrolimus-FKBP12 complexes to calcineurin inhibits phosphatase activity of calcineurin. This inhibition then suppresses the transcription of IL-2 via inhibition of the dephosphorylation and impaired translocation of the nuclear factor of activated T cells (NFAT).[10,11,12] This in turn regulates IL-2 transcription and thus T cell activation.[13,14,15] Calcineurin and NFAT isoforms are, however, not T cell specific and inhibition of this pathway by cyclosporine and tacrolimus gives rise to toxicity beyond immunosuppression.[16,17,18,19,20] The immediate and long-term nephrotoxic effects of cyclosporine and tacrolimus are a major concern in patients undergoing transplant procedures.[21,22,23,24]

While on one hand, vascular, tubular and thrombotic microangiopathy are the dominant factors in the immediate post-transplant period; tubulo-interstitial and glomerular effects predominate on prolonged use. However, though a considerable amount of data is available on the toxicity profile of CNIs in patients of solid organ transplant, the review of recipients post HSCT is very limited worldwide and particularly, in India. Interplay of additional factors (intracellular incorporation of electrolytes in the newly forming hematopoetic cells, abnormal mineral bone metabolism etc) influence the metabolic and renal profile in this group of patients (recipients of HSCT) and make it even more complex. Maintaining the concentrations of the CNIs cyclosporine and tacrolimus within preset target ranges is very complicated because of their high inter- and intraindividual pharmacokinetic variability, resulting

in very low correlation of their dose and concentration. This unpredictable dose–concentration relationship results from a high variability in absorption, distribution, metabolism and elimination of these compounds.

With this background, we reviewed the ionic status and renal profile in patients on CNIs (Cyclosporine/ Tacrolimus) post-HSCT in a tertiary care hospital in India. A co-relation was done to analyse the effect of the dose of CNI on the affected electrolyte. The importance of periodic evaluation of serum electrolyte level as a part of diagnostic and therapeutic protocol in recipients of HSCT on CNIs was also explored.

MATERIALS AND METHODS

31 patients who underwent HSCT over a period of 4 months (February- June 2017) were included in the study. The study group included patients who were on treatment with calcineurin inhibitors, either cyclosporine or tacrolimus, after HSCT. Plasma samples were collected in EDTA vials for cyclosporine/ tacrolimus, which were analysed in Architect i1000 by CMIA (Chemiluminescent Microparticle Immunoassay). 2 levels of control were run before the estimation of samples. The detection range of Cyclosporine was 30-1500 ng/mL and Tacrolimus was 2-30 ng/mL. Serum sample were estimated weekly for serum Magnesium, Potassium & Creatinine, to evaluate the effect of the CNIs on the respective parameters. Serum Magnesium, Potassium & Creatinine were analysed on Roche C-6000 autoanalyser by Xylidyl- Blue Diazonium, ISE- Indirect & Jaffe's Kinetic method respectively.

The Biological Reference Interval (according to age)

Potassium
3.5-5.1 mmol/L

Magnesium
5 months-6 years: 0.70-0.95 mg/dL
6-12 years: 0.70-0.86 mg/dL
12-20 years: 0.70-0.91 mg/dL

Adults
0.66-1.07 mg/dL
60-90 years: 0.66-0.99 mg/dL

Creatinine
1-<3 y: 0.24- 0.41 mg/dL
3-<5 y: 0.31-0.47 mg/dL
5-<7 y: 0.32-0.59 mg/dL
7-<9 y: 0.40-0.60 mg/dL
9-<11 y: 0.39-0.73 mg/dL
11-<13 y: 0.53-0.79 mg/dL
13-<15 y: 0.57-0.87 mg/dL

Adult
Male: 0.70-1.20 mg/dL
Female: 0.50-0.90 mg/dL

Cyclosporine (Trough Level)
12 hrs after dose 100 - 400 ng/ml
24 hrs after dose 100 - 200 ng/ml
Toxic Range > 400 ng/ml

Tacrolimus: 5-15 ng/ml

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been done in compliance with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration. It has been approved by the authors' institutional Ethical Committee.

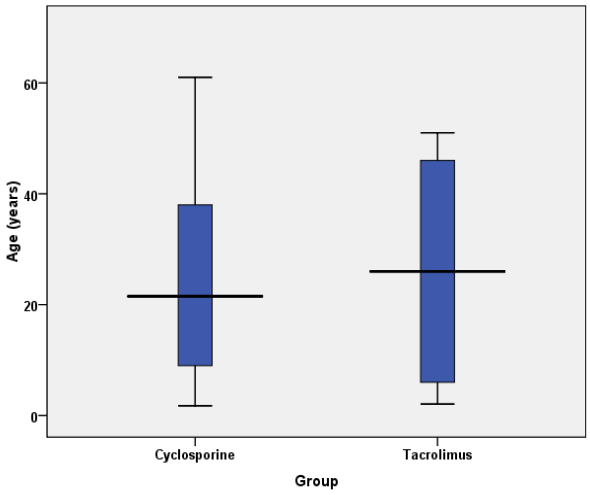
Statistical Analysis

Statistical analysis was performed by the SPSS program for Windows, version 17.0(SPSS, Chicago, Illinois). Continuous variables are presented as mean ± SD, median (IQR) and categorical variables are presented as absolute numbers and percentage. Data were checked for normality before statistical analysis. Normally distributed continuous variables were compared using the unpaired t test, whereas the Mann-Whitney U test was used for those variables that were not normally distributed. Categorical variables were analyzed using either the chi square test or Fisher's exact test. Friedman test was used for various values over a period of 4 weeks. For all statistical tests, a p value less than 0.05 was taken to indicate a significant difference.

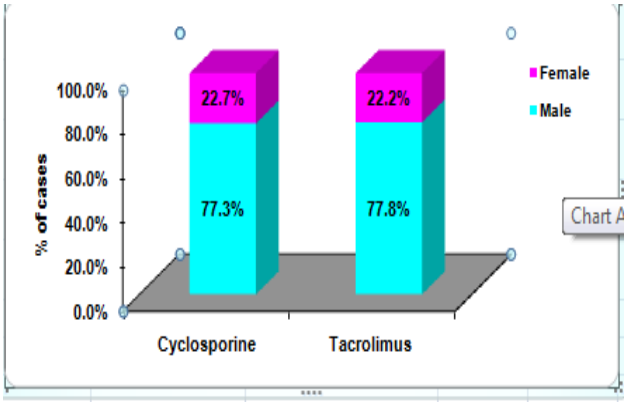
RESULTS

A total of 31 patients who underwent HSCT between February and June 2017 were included in this retrospective study. 22 patients (71%) were on treatment with cyclosporine while 9 (29%) were given tacrolimus. The median age of patients on cyclosporine was 21.50 (IQR: 8.75 - 38.25) and that of patients on tacrolimus was 26.00 (IQR: 5.29 - 47.50). The gender distribution of the patients on cyclosporine (22.7% female & 77.3% male) and tacrolimus (22.2% female & 77.8%) was also compared statistically. The age and sex distribution in the 2 groups of CNI was comparable (Graph I&II). The data available for serum magnesium, potassium and creatinine levels in the 3 weeks post transplant were evaluated further. Age appropriate biological reference intervals were taken into consideration. Data obtained from patients on cyclosporine evaluated every week after transplant for 3 weeks show a significant decrease in serum magnesium level in the 3rd week (mean values being 1.69, 1.42, 1.23 respectively) (p value: < 0.05) (Table I & Graph III). In patients on tacrolimus however the decrease is statistically insignificant (Table IV). The correlation between the dose of cyclosporine and serum magnesium level was found to be statistically significant in the 3rd week post transplant (Table VII & Graph IV). This observation assumes significance because the mean magnesium level also shows maximum decline in the 3rd week. However, there is no significant correlation

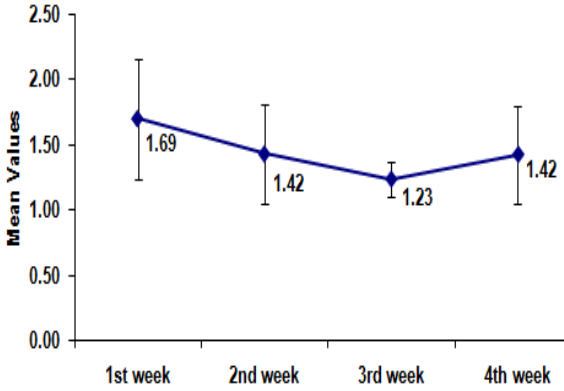
between the dose of tacrolimus and serum magnesium level (Table VIII). Serum creatinine and potassium levels were not significantly altered in the post-transplant period after initiation of the calcineurin inhibitors (Table II, III, V, VI).



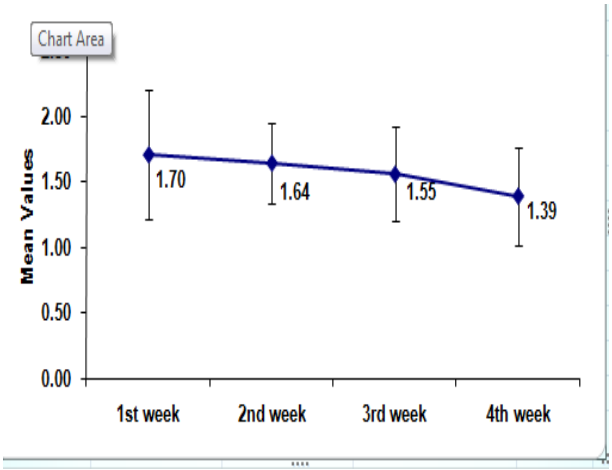
Graph I: Age-wise distribution of patients on CNI (Cyclosporine / Tacrolimus).



Graph II: Sex-wise distribution of patients on CNI (Cyclosporine / Tacrolimus).



Graph III: Distribution of Serum Magnesium level over 4 weeks post transplant in patients on Cyclosporine ($p<0.05$, significant).



Graph IV: Distribution of Serum Magnesium level over 4 weeks post transplant in patients on Tacrolimus.

Table I: Distribution of Serum Magnesium level over 3 weeks post transplant in patients on Cyclosporine.

Serum Magnesium levels	Mean	Std. Deviation	N	p value
1st week	1.69	0.46	9	0.017
2nd week	1.42	0.38	9	
3rd week	1.23	0.13	9	

Table II: Distribution of Serum Creatinine level over 3 weeks post transplant in patients on Cyclosporine.

Serum Creatinine levels	Mean	Std. Deviation	N	p value
1st week	0.48	0.41	10	0.150
2nd week	0.50	0.33	10	
3rd week	0.49	0.39	10	

Table III: Distribution of Serum Potassium level over 3 weeks post transplant in patients on Cyclosporine.

Potassium	Mean	Std. Deviation	N	p value
1st week	3.67	0.345	15	0.719
2nd week	3.77	0.475	15	
3rd week	3.73	0.452	15	

Table IV: Distribution of Serum Magnesium level over 3 weeks post transplant in patients on Tacrolimus.

Serum Magnesium levels	Mean	Std. Deviation	N	p value
1st week	1.70	0.49	7	0.513
2nd week	1.64	0.31	7	
3rd week	1.55	0.36	7	

Table V: Distribution of Serum Creatinine level over 3 weeks post transplant in patients on Tacrolimus.

Serum Creatinine levels	Mean	Std. Deviation	N	p value
1st week	1.29	2.11	7	0.409
2nd week	0.51	0.35	7	
3rd week	0.67	0.54	7	

Table VI: Distribution of Serum Potassium level over 3 weeks post transplant in patients on Tacrolimus.

Potassium	Mean	Std. Deviation	N	p value
1st week	4.04	0.34	6	0.450
2nd week	4.19	0.57	6	
3rd week	3.84	0.76	6	

Table VII: Correlation of the dose of Cyclosporine with Serum Magnesium level.

Dose	Magnesium			
	1st week	2nd week	3rd week	4th week
1st week	-0.238			
	0.285			
2nd week		0.047		
		0.85		
3rd week			-0.620	
			0.014	

Table VIII: Correlation of the dose of Tacrolimus with Serum Magnesium level.

Dose	Magnesium			
	1st week	2nd week	3rd week	4th week
1st week	-0.217			
	0.576			
2nd week		-0.317		
		0.406		
3rd week			-0.086	
			0.872	

DISCUSSION AND CONCLUSION

Haematopoietic stem cell transplantation has gained worldwide acceptance as a therapeutic option for multiple haematological and non-haematological conditions. Calcineurin inhibitors cyclosporine and tacrolimus (FK 506) now form the cornerstone of immunosuppressive regimens following bone marrow transplantation. Several studies have analysed the effect of calcineurin inhibitors on the renal function and electrolyte levels of patients undergoing solid organ transplant. However, the effect of CNIs on the serum electrolyte and creatinine level of recipients of bone marrow transplant (BMT) remains widely unexplored. The incidence and timing of these abnormalities is also largely unknown. The present study aims at exploring the serum ionic status (magnesium and potassium) and creatinine level in recipients of BMT on CNI therapy. This study assumes greater significance because there is limited data worldwide, and none from the Indian subcontinent in this area in recipients of BMT.

In this retrospective study conducted in a tertiary care hospital in India, considering the biological reference interval appropriate for the age of the patient, 20/31 (64.5%) patients of BMT on either cyclosporine or tacrolimus had a low level of magnesium in the immediate post-transplant period. 7/31 patients (22.5%) had a magnesium level of less than one at least once during the same period. The magnesium level decreased significantly over a 3 week period in patients receiving cyclosporine, with the maximum decline seen in the third week post transplant (p value:0.017). A significant correlation was also observed between serum cyclosporine level and magnesium in the third week (p value: 0.014). June CH and Kone BC observed a similar hypomagnesemia in 45.8% and 88% of the recipients of BMT.[25,26] Our study also corroborates with the findings of J H Lee and Philibert D, where hypomagnesemia occurred in 16.7% and (66.6%) of the patients on cyclosporine.[27,28] In the present study, hypokalemia occurred in 15/31(48.3%) recipients of BMT on CNIs. Only 1 patient had a single episode of hyperkalemia. Similar studies observed that hypokalemia occured in 47.9% and 4% of all recipients of BMT respectively.[27,28]

Limited information is available about the correlation of CNIs with ionic status in patients of bone marrow transplant. Lesser still is the data comparing the effects of the two commonly used CNIs cyclosporine and tacrolimus.

In a prospective study, 41 marrow transplant patients were evaluated to analyse the possible association of hypomagnesemia with cyclosporine. Eleven of 24 patients receiving cyclosporine versus 1 of 14 patients receiving methotrexate had magnesium levels less than 1mEq/L, or were begun on replacement therapy for presumed symptomatic hypomagnesemia. It was concluded that renal magnesium wasting may be added to the spectrum of nephrotoxicity resulting from cyclosporine and several adverse reactions previously attributed to cyclosporine may be secondary to magnesium deficiency.[25] In another case-control study, 32 patients who became hypertensive on treatment with cyclosporine were matched with 32 cyclosporine-treated

controls. At the time of development of hypertension, the hypertensive patients had a mean (+/- SD) Mg of 1.22 +/- 0.20 mEq/L versus controls 1.40+/-0.33 mEq/L (P less than 0.01). The study indicated that hypertension and hypomagnesemia are coincident toxicities in cyclosporine-treated patients and that acquired derangements in magnesium metabolism may contribute to the development of hypertension.[29] In another study, recipients of BMT either on cyclosporine or cyclophosphamide therapy developed significant hypomagnesemia, with the incidence being higher with the use of the former. The incidence of diastolic hypertension and hypomagnesemia was greater in patients treated with cyclosporine.[26] 36 patients (12 on cyclosporine, 24 on tacrolimus) were evaluated for serum Mg, which showed a decline in both groups in the first week after HSCT. Although both CNIs increased urinary Mg excretion and caused hypomagnesemia shortly after HSCT, the effect was more significant with tacrolimus than with CSA.[30] However, our study differs in this area, observing a significant decrease in magnesium over a period of three weeks in patients on cyclosporine (p value: <0.05). The dose of cyclosporine also correlated significantly with the magnesium level in the third week (p value: <0.05). The change in magnesium level over the same period in patients on tacrolimus was however not statistically significant. Since the level of creatinine or potassium was not significantly altered in patients on CNIs, the hypomagnesemia may be reflective of the independent effect of the drugs on renal tubules, resulting in altered transport of magnesium through the tubular cells, and hence, magnesium wasting. However, a limitation of this study, as in similar studies on this subject in the past, was the small sample size available for analysis.

In a study conducted in Korea, laboratory data was collected for 311 patients with a median age of 32 years and the frequency of abnormalities detected within 100 days after HSCT was hyperkalemia (8.0%), hypokalemia (47.9%), hypermagnesemia (2.9%) and hypomagnesemia (16.7%).[27] Another study analysed the incidence and timing of electrolyte abnormalities in 48 patients following autologous HSCT and detected hypokalaemia in 81% and hypomagnesaemia in 67% of the patients. However, high levels of electrolytes occurred much less frequently. Hyperkalaemia occurred in 4% and hypermagnesemia in 10% of the recipients.[28] Since studies have shown that the occurrence of severe metabolic abnormalities within 100 days after allogeneic HCT was significantly associated with inferior clinical outcomes, more research is required in this direction.[27]

The precise mechanism by which CNIs cause Mg wasting is not clear. Unlike other ions that are primarily reabsorbed in the proximal tubules, the majority of Mg is reabsorbed in the more distal part of the renal tubules. The thick limb of the loop of Henle accounts for a large fraction of renal Mg reabsorption and the distal tubule plays an important role in the final adjustment of renal Mg excretion. Therefore, the loop of Henle and/or distal tubule could be the likely sites where cyclosporine and tacrolimus inhibit renal Mg reabsorption.[31,32]

The underlying mechanisms explaining low electrolyte levels in recipients of HSCT seem to be multifactorial and can be grouped into five categories: reduction of intake, enhanced gastrointestinal loss, intracellular incorporation of electrolytes into the new forming haematopoietic cells, enhanced renal loss and abnormal mineral bone metabolism.[28,33,34,35]

Some of the effects of cyclosporine and tacrolimus on tubular function can be explained by reduced expression of the Na_-K_-2Cl--cotransporter (NKCC2) at the apical membrane of tubular epithelial cells.[36,37] The hyperkalemia seen with calcineurin inhibition is probably due to the inhibitory effects on Na-K-ATPase in collecting ducts and possibly, distal tubular acidosis.[36,37,38,39,40] In addition, there is evidence that decreased numbers of mineralocorticoid receptors, detected in 75% of patients treated with cyclosporine in renal transplants, lead to hyperkalemia and metabolic acidosis as a result of aldosterone resistance.[41] Recently, it was demonstrated that cyclosporine reduced paracellin-1 expression in thick ascending limb cells.[42] The resulting decrease in magnesium transport likely contributed to the magnesium wasting and hypomagnesemia induced by cyclosporine.

Most of the studies mentioned above were performed in patients of solid organ transplant (non-HSCT) on CNI therapy and hence, their incidence in recipients of HSCT needs further evaluation. It is however compounded by additional factors specific to HSCT, which makes it even more complex. In this group of patients, volume expansion is first used before haematopoietic stem cell reinfusion in order to prevent haemolysis-induced renal failure. The natriuresis that ensues is accompanied by an increased excretion of potassium, calcium and a reduction in proximal phosphate reabsorption.[43] Loop diuretics are commonly used to reduce the fluid overload. This practice further enhances the renal loss of potassium, calcium and magnesium.[44,45] Finally, reduced levels of some electrolytes may play an important role in the renal loss of other electrolytes.

In the present study, 8/31(25.8%) recipients of HSCT on CNIs had an elevated creatinine level. There was no significant correlation between the dosage of cyclosporine/ tacrolimus and serum creatinine level. Studies showed that patients treated with CNIs were at high risk of developing renal injury.[46] Calcineurin inhibitor nephrotoxicity (CIN) was manifested either as acute azotemia, which was largely reversible after reducing the dose, or as chronic progressive renal disease, which was usually irreversible.[47,48,49] A similar pattern of renal injury was seen with tacrolimus, thereby suggesting a drug class effect. In a study conducted in 1983 in the first 100 days post transplant, renal

dysfunction was much less frequent in the recipients of MTX than in those on cyclosporine.[50]

The onset of acute kidney injury (elevated levels of serum creatinine up to 100 days after transplantation) and chronic kidney disease (elevated levels at or after 100 days) affect 10 to 70% of transplant recipients.[51,52,53] The low incidence of altered creatinine level in our study group (25.8%) may be explained by the fact that the patients were evaluated in the immediate post-transplant period, much before any effect could be manifested on the glomeruli. Moreover, it indicates that sepsis and other comorbid conditions, if prevented or controlled well, the risk of acute injury post transplant, remains minimal. Most of the research in this area has been conducted in recipients of solid organ transplant. The situation in recipients of HSCT is however further complicated by BMT-specific reasons like marrow infusion toxicity, hepatic veno-occlusive disease, thrombotic microangiopathy and graft versus host disease.[54] Some studies suggest that reducing the dose of CNIs, or using protocols without calcineurin inhibition may ultimately minimize the risk of drug toxicity. New experiences with non-nephrotoxic agents and protocols allow for early calcineurin inhibitor reduction or elimination without increasing the risk of allograft rejection.[24] However, they are still under evaluation.

In conclusion, it may be said that the present study observed a significant decrease in serum magnesium level over three weeks in the immediate post-transplant period in recipients of HSCT administered cyclosporine. The extent of hypomagnesemia and its correlation with the dose of cyclosporine in the 3rd week post transplant was a significant finding of this study. Serum potassium and creatinine levels however, did not show any significant alteration during the same period with either cyclosporine or tacrolimus. We therefore conclude that frequent and regular monitoring of serum magnesium level may be useful in preventing co-morbidities in recipients of HSCT and facilitate better engraftment.

It should therefore, be included as an integral part of therapeutic protocols. This study being a first of its kind from the Indian subcontinent, and one of the very few studies conducted on recipients of HSCT, assumes greater significance. The effect of calcineurin inhibition on recipients of HSCT being multifactorial, is likely to be more complex, and hence, needs to be explored further.

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Conflict of interest: The authors state no conflict of interest.

REFERENCE

1. Calne RY, Rolles K, White DJ, Thiru S, Evans DB, McMaster P et al. Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs: 32 kidneys, 2 pancreases and 2 livers. Lancet, 1979; 2: 1033–1036.
2. Calne RY, White DJ, Thiru S, Evans DB, McMaster P, Dunn DC et al. Cyclosporin A in patients receiving renal allografts from cadaver donors. Lancet, 1978; 2(8104-5): 1323-7.
3. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramman R, Jain A. FK 506 for liver, kidney, and pancreas transplantation. Lancet, 1989; 2: 1000–1004.
4. Fung JJ, bu-Elmagd K, Todo S, Shapiro R, Tzakis A, Jordan M et al. Overview of FK506 in transplantation. Clin Transpl, 1990; 115–121.
5. Andreoni KA, Brayman KL, Guidinger MK, Sommers CM. Calcineurin Inhibitor Nephrotoxicity. Clin J Am Soc Nephrol. 2009; 4: 481–508.
6. Takahashi N, Hayano T, Suzuki M. Peptidyl-prolyl cistrans isomerase is the cyclosporin A-binding protein cyclophilin. Nature, 1989; 337: 473–475.
7. Fischer G, Wittmann-Liebold B, Lang K, Kiefhaber T, Schmid FX. Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. Nature, 1989; 337: 476–478.
8. Harding MW, Galat A, Uehling DE, Schreiber SL. A receptor for the immunosuppressant FK506 is a cis-trans peptidyl- prolyl isomerase. Nature, 1989; 341: 758–760.
9. Siekierka JJ, Hung SH, Poe M, Lin CS, Sigal NH. A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. Nature, 1989; 341(6244): 755-7.
10. Flanagan WM, Corthesy B, Bram RJ, Crabtree GR. Nuclear association of a T-cell transcription factor blocked by FK-506 and cyclosporin A. Nature, 1991; 352: 803–807.
11. Jain J, McCaffrey PG, Miner Z, Kerppola TK, Lambert JN, Verdine GL et al. The T-cell transcription factor NFATp is a substrate for calcineurin and interacts with Fos and Jun. Nature, 1993; 365: 352–355.
12. Shaw KT, Ho AM, Raghavan A, Kim J, Jain J, Park J et al. Immunosuppressive drugs prevent a rapid dephosphorylation of transcription factor NFAT1 in stimulated immune cells. Proc Natl Acad Sci U S A., 1995; 92(24): 11205-9.
13. Clipstone NA, Crabtree GR. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. Nature, 1992; 357: 695–697.
14. O'Keefe SJ, Tamura J, Kincaid RL, Tocci MJ, O'Neill EA. FK-506- and CsA-sensitive activation of the interleukin-2 promoter by calcineurin. Nature, 1992; 357: 692–694.
15. Emmel EA, Verweij CL, Durand DB, Higgins KM, Lacy E, Crabtree GR. Cyclosporin A specifically

inhibits function of nuclear proteins involved in T cell activation. Science, 1989.
16. Su Q, Zhao M, Weber E, Eugster HP, Ryffel B. Distribution and activity of calcineurin in rat tissues. Evidence for post-transcriptional regulation of testis-specific calcineurin B. Eur J Biochem, 1995; 230(2): 469-74.
17. Rao A, Luo C, Hogan PG. Transcription factors of the NFAT family: Regulation and function. Annu Rev Immunol, 1997; 15: 707–747.
18. Halloran PF, Helms LM, Kung L, Noujaim J. The temporal profile of calcineurin inhibition by cyclosporine in vivo. Transplantation, 1999; 68: 1356–1361.
19. Kung L, Batiuk TD, Palomo-Pinon S, Noujaim J, Helms LM, Halloran PF. Tissue distribution of calcineurin and its sensitivity to inhibition by cyclosporine. Am J Transplant, 2001; 1: 325–333.
20. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M. Cyclosporine-associated chronic nephropathy. N Engl J Med., 1984; 311: 699–705.
21. Palestine AG, Austin HA III, Balow JE, Antonovych TT, Sabnis SG, Preuss HG et al. Renal histopathologic alterations in patients treated with cyclosporine for uveitis. N Engl J Med., 1986; 314: 1293–1298.
22. Ojo AO, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW et al. Chronic renal failure after transplantation of a nonrenal organ. N Engl J Med., 2003; 349: 931–940.
23. Olyaei AJ, de Mattos AM, Bennett WM. Nephrotoxicity of immunosuppressive drugs: New insight and preventive strategies. Curr Opin Crit Care., 2001; 7: 384–389.
24. June CH, Thompson CB, Kennedy MS, Nims J, Thomas ED. Profound hypomagnesemia and renal magnesium wasting associated with the use of cyclosporine for marrow transplantation. Transplantation, 1985; 39(6): 620-4.
25. Kone BC, Whelton A, Santos G, Saral R, Watson AJ. Hypertension and renal dysfunction in bone marrow transplant recipients. Q J Med., Dec, 1988, 69(260): 985-95.
26. J-H Lee, S-J Choi, J-H Lee, S-E Kim, M Seol, Y-S Lee et al. Severe metabolic abnormalities after allogenic hematopoetic cell transplantation. Bone Marrow Transplant, 2005; 35: 63-9.
27. Philibert D, Desmeules S, FilionA, Poirierm and Agharazii M. 2008. Incidence and severity of early electrolyte abnormalities following autologous haematopoietic stem cell transplantation. Nephrol Dial Transplant. 23: 359–363.
28. June CH, Thompson CB, Kennedy MS, Loughran TP Jr, Deeg HJ. Correlation of hypomagnesemia with the onset of cyclosporine-associated hypertension in marrow transplant patients. Transplantation, 1986; 41(1): 47-51.
29. Aisa Y, Mori T, Nakazato T, Shimizu T, Yamazaki R, Ikeda Y et al. Effects of immunosuppressive agents on magnesium metabolism early after allogeneic hematopoietic stem cell transplantation. Transplantation, 2005; 80(8): 1046-50.
30. Cole DA, Quamme GA. Inherited disorders of renal magnesium handling. J Am Soc Nutr, 2000; 11: 1937-47
31. Mazzola BL, Vannini SDP, Truttman AC, et al. Long-term calcineurin inhibition and magnesium balance after renal transplantation. Transpl Int., 2003; 16: 76-81.
32. James GWIII, Abbott LD Jr. Metabolic studies in pernicious anemia. Metabolism, 1952; 52: 259–270.
33. Lawson DH, Murray RM, Parker JL. Early mortality in the megaloblastic anaemias. Q J Med., 1972; 41: 1–14.
34. Schiffman FJ, Schiffman RL, Rosa RM. Cellular proliferation and hypokalemia. Ann Intern Med., 1977; 87: 635–636.
35. Lea JP, Sands JM, McMahon SJ, Tumlin JA. Evidence that the inhibition of Na/K-ATPase activity by FK506 involves calcineurin. Kidney Int., 1994; 46: 647–652.
36. Deppe CE, Heering PJ, Tinel H, Kinne-Saffran E, Grabensee B, Kinne RK. Effect of cyclosporine A on Na/ K-ATPase, Na/K/2Cl- cotransporter, and H/ K -ATPase in MDCK cells and two subtypes C7 and C11. Exp Nephrol, 1997; 5: 471–480.
37. Stahl RA, Kanz L, Maier B, Schollmeyer P. Hyperchloremic metabolic acidosis with high serum potassium in renal transplant recipients: A cyclosporine A associated side effect. Clin Nephrol, 1986; 25: 245–248.
38. Tumlin JA, Sands JM. Nephron segment-specific inhibition of Na_/K-ATPase activity by cyclosporin A. Kidney Int., 1993; 43: 246–251.
39. Younes-Ibrahim M, Barnese M, Burth P, Castro-Faria MV. Inhibition of purified human kidney Na-K-ATPase by cyclosporine A: A possible mechanism for drug human nephrotoxicity. Ann N Y Acad Sci., 2003; 986: 633–635.
40. Heering PJ, Kurschat C, Vo DT, Klein-Vehne N, Fehsel K, Ivens K. Aldosterone resistance in kidney transplantation is in part induced by a down-regulation of mineralocorticoid receptor expression. Clin Transplant, 2004; 18(2): 186-92.
41. Chang CT, Hung CC, Tian YC, Yang CW, Wu MS. Ciclosporin reduces paracellin-1 expression and magnesium transport in thick ascending limb cells. Nephrol Dial Transplant, 2007; 22: 1033–40.
42. Suki WN, Martinez-Maldonado M, Rouse D, Terry A. Effect of expansion of extracellular fluid volume on renal phosphate handling. J Clin Invest, 1969; 48: 1888–94.
43. White MG, van Gelder J, Eastes G. The effect of loop diuretics on the excretion of Naþ, Ca2, Mg2þ, and Cl-. J Clin Pharmacol, 1981; 21: 610–14.
44. Di tefano A, Roinel N, de Rouffignac C, Wittner M. Transepithelial Ca2þ and Mg2þ transport in the cortical thick ascending limb of Henle's loop of the mouse is a voltage-dependent process. Ren Physiol Biochem, 1993; 16: 157–66.

45. Burdmann EA, Andoh TF, Yu L, Bennett WM. Cyclosporine nephrotoxicity. Semin Nephrol, 2003; 23: 465.
46. Kahan BD. Cyclosporine. N Engl J Med., 1989; 321: 1725.
47. Kopp JB, Klotman PE. Cellular and molecular mechanisms of cyclosporin nephrotoxicity. J Am Soc Nephrol, 1990; 1: 162.
48. de Mattos AM, Olyaei AJ, Bennett WM. Nephrotoxicity of immunosuppressive drugs: long-term consequences and challenges for the future. Am J Kidney Dis., 2000; 35: 333.
49. Atkinson K, Biggs JC, Hayes J, Ralston M, Dodds AJ, Concannon AJ et al. Cyclosporin A associated nephrotoxicity in the first 100 days after allogeneic bone marrow transplantation: three distinct syndromes. Br J Haematol, 1983; 54(1): 59-67.
50. Higgins RM, Hart P, Lam FT, Kashi H. Conversion from tacrolimus to cyclosporin in stable renal transplant patients: safety, metabolic changes, and pharmacokinetic comparison. Transplantation, 2000; 70: 199.
51. Margreiter R. Efficacy and safety of tacrolimus compared with cyclosporin microemulsion in renal transplantation: a randomised multicentre study. Lancet, 2002; 359: 741.
52. Cho BS, Yahng SA, Lee SE, et al. Validation of recently proposed consensus criteria for thrombotic microangiopathy after allogeneic hematopoietic stem-cell transplantation. Transplantation, 2010; 90: 918-26.
53. Kemmner S, Verbeek M, Heemann U. Renal dysfunction following bone marrow transplantation. J Nephrol, 2017; 30(2): 201-9.