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Spot light on C_2 as abbreviated alogrithm for safe and effective therapeutic monitoring of cyclosporine in renal transplant

Loan Gh. Mohd.*, [1], Wafai Z.A.*, Reshi Abdul Rashid[2]
*,[1] Department of Clinical Pharmacology, Sheri Kashmir Institute of Medical Sciences, Srinagar, J&K, India
[2]Department of Nephrology, Sheri Kashmir Institute of Medical Sciences, Srinagar, J&K, India

*Dr. G M Loan, Dr. Resident Department of Clinical Pharmacology SKIMS, Soura J&K, India, 190011
loneskims@rediffmail.com

Abstract

Whole blood trough level (C_0) has been traditionally used for Cyclosporine (CsA) dosage adjustments. Absolute C_0 susceptible to extensive variability from toxicity to immunogenic response may not be a dependable guide for adjustment. Prospective studies in renal transplant suggest that CsA dosing based on Two Hour Peak (C_2) results in less rejection and better renal function that C_0. However, ideal TDM for CsA has yet to be defined. With the objective that large across-the-day variation of CsA bioavailability due to circadian and food effects observed even at average steady state, can be incorporated into meaningful monitoring by using a normalized-dose adjusted C_2/ C_0 algorithm accounting for both the drug absorption and elimination, this study using EMIT assay was conducted to assess C_2/C_0 ratio as abbreviated *PK* model that could predict rejection and renal function status and help develop guidelines for better CsA TDM. Follow up patients (n=35: male 29, female 6) at varied post-renal transplant period (6 months to 11 years), age (18 to 85 years) and weight (60 to 78 Kg) receiving Cyclosporine 75-200mg daily in 12 hourly increments that were equal in 20 and unequal in 15 patients in which evening dose 83.9±21.2mg was significantly higher ($p< 0.001$) than morning dose 74.8±24.6mg. C_0 and C_2 at steady state were 194±74.6ng/ml and 1018.7±278.9ng/ml respectively. C_2/C_2 was 5.6±1.9 with optimal renal function (Sr. creatinine 1.4±1.2mg/dl; Blood Urea 33.5±6.0mg/dl; Uric Acid 5.5±0.7mg/dl; systolic BP 131.8±8.9, diastolic BP 88.5±6.2mmHg and Hb 12.6±1.2g%). Other than direct correlation as expected with C_0, C_2/C_0 ratio was independent of subjecdt and treatment variables. At 95% C.I. for projected population the C_2/C_0 ratio was within 5.0 to 6.3. The ratio did not show any difference in spite of different sets of C_0 and C_2 values. In conclusion, C_2/C_0 may have the potential of an algorithm for monitoring safety and predict risk of cyclosporine immunosuppression following renal allograft.

Key Words: CsA, C2/C0, TDM, AUC.

Introduction

Calcineurin inhibitor Cyclosporine (C_sA), a cyclic polypeptide consisting 11 aminoacids has revolutionized the immunosuppression and contributed significantly to the success of maintaining long term function of renal allografts. "Therapeutic Drug Monitoring" is an integral part of transplant protocols but has required continual refinement. Immunosuppressants require therapeutic monitoring because of their narrow therapeutic index, significant individual variation [1] and food effects [2]. Trough levels (C_0) were measured for the last 20 years but over the time were realized to be less than optimal as an index of C_sA exposure for dosage individualization [3]. TDM is employed to measure blood drug levels so that the most effective dosage can be determined and toxicity prevented. C_0 is a poor predictor of graft exposure to Cyclosporine and C_2 reflects this exposure more exactly [4]. C_2 is more accurate single-sample marker for all C_{0-4} than C_0 alone. Considering high frequency of Cyclosporine hepatotoxicity and neurotoxicity, the target levels of C_0 and C_2 in living donor transplantation should be lower [5]. Many clinicians monitor Cyclosporine blood levels only when a clinical event (e.g. renal dysfunction or rejection) occurs. In that setting either C_0 or C_2 levels help to ascertain whether inade3quate immunosuppression or drug toxicity is present. It has been shown repeatedly that C_0 concentration does not reflect the area under the curve (AUC) for Cyclosporine exposure in individual patients. A practical approach is to measure the overall exposure of a patient to the drug by taking the level at 2^{nd} hour (C_2) after subsequent dose administration [6]. C_2 is predictive for acute rejection and not of chronic rejection. C_2 is expected to provide a potentially important reduction in the risk of acute rejection without increasing the estimated cost of care in the first year post-transplant [6].

Organ transplantations require lifelong immunosuppressive therapy that prevents allograft rejection. TDM of cyclosporine in maintenance therapy can aid in titration of drug doses to the individual needs, thus avoiding adverse drug reactions which are a direct consequence of patient variability in drug disposition [7]. It has been seen that C_0 has a more predictive correlation with serum cholesterol after renal transplant in adolescent patients [8]. Bioavailability of Microemulsion CsA formation "Neoral R" is less dependent on food intake and bile secretion, thus offering advantage of reduced variability [9]. C_2 monitoring is now being widely adopted as an accurate and practical measure of drug exposure and can be combined with pharmacodynamics to optimize immunosuppression [10]. So it is not certain as to which level (C_0 or C_2) is appropriate in a transplant patient that gives good correlation with the pharmacodynamic benefits though C_2 monitoring is being adopted as a practical measure of drug exposure combined with pharmacodynamic methods to optimize immunosuppression [11]. So this study approves both the levels like C_2/C_0 for the correlation of pharmacodynamic response.

Material Methods

One and a half year prospective study included 35 maintenance renal transplant recipients (29 males; 6 females) at varied post-transplant period (6 months to 11 years) and ranging in age from 18 to 65 years who were selected from patients attending the Department of Clinical Pharmacology between March 2006 and August 2007. Chronic End Stage Renal Disease as diabetic complication was excluded. The patients, who were recruited in the study, were additionally taking same immunosuppressants like azothoprine, mycophenolate mofetil and corticosteroids. Patients were selected on the basis of appropriate individual pharmacodynamic responses with Cyclosporine (Neoral *Avantis*) irrespective of dosage and/or dosing intervals. KFT and lipid profile were measured on the days of recruitment of patients. Patients on 12 hoursly incremental CsA dose schedule for at least preceding 8 weeks reported after overnight fasting. Blood sample was drawn just before morning CsA dose at 9.00 A.M. for C_0 and then 2 hours after taking the morning CsA

dose for C_2 levels. Customary breakfast was allowed at 10.00 A.M. All other maintenance medicines were provided as usual according to schedule. Blood pressure was recorded before collection of blood samples. The whole blood samples were collected in EDTA vials for C_0, C_2 and in caped tubes for serum chemistry. Quantitative analysis for C_0 and C_2 was done pre-validated EMIT assay using ERBA Chem. Pr. Analyzer. Samples for C_2 levels were diluted as per the protocol. Lyphocheck Cyclosporine levels 2 and 3 were used as controls. Data were summarized and statistical analysis was done using SPSS Statistical Software. Standard descriptive statistics and dependant t-test were used when analyzing relationship between immunosuppression and renal function and between C_0, C_2 and C_2/C_0 alogrithm.

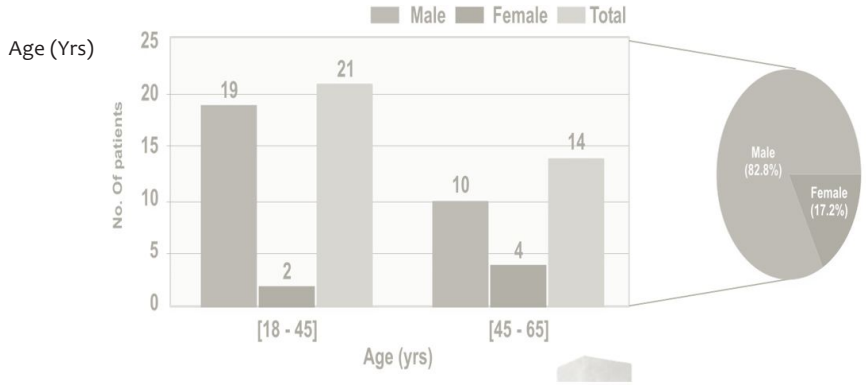
Results

From total number of renal transplant follow-up patients during study period, 35 patients without history of primary graft dysfunction or frequent dose adjustments that would have attained satisfactory CsA steady state were included.

Table 1 provides salient features of patient presentation. Their mean age was 41.2±13.1 (18-65) years; males predominating (n=26; 82.8%) and majority (66%) being on younger side at 18-45 years (Fig. 1).

TABLE 1. Clinical Presentation of 35 Renal Transplant Patients

	Mean (SD)	Minimum	Maximum
Age (Yrs)	41.14 (13.113)	18.0	65.0
Morning dose	156.43 (45.107)	50.0	200.0
Weight (kgs)	66.63 (6.353)	45.0	78.0
Hb%	12.677 (1.2322)	10.0	14.7
SBP	131.86 (8.918)	110.0	150.0
DBP	88.54 (6.294)	80.0	104.0
Serum urea (mg/dl)	33.57 (6.079)	24.0	45.0
Serum creatinine (mg/dl)	1.413 (0.4050)	1.0	2.8
TGs (mg/dl)	207.14 (22.729)	180.0	250.0
Uric acid (mg/dl)	5.503 (0.7637)	4.8	7.3
Transplant period (Yrs)	3.02 (2.293)	00.00.00	12.0



For all patients, donors were live relatives. The incremental morning dose was 156.4±45.1 (50-200mg) irrespective of transplant period varying from 6 months to 13 years and the average renal functions were maintained normal as indicated by serum creatinine (1.4±0.4mg/dl), blood urea (33.5±6.0mg/dl), uric acid (5.5±0.8mg/dl), SBP/DBP (131.8±8.9/88.5±6.2mmHg) and hemoglobin (12.7±1.2%). The CsA exposure at trough i.e. C_0 was 194.1±76.8 (72-400) ng/ml and at 2 hour peak i.e. C_2 was 1018.7±278.9 (555-1950) ng/ml during the course of maintenance treatment in patients at steady state. C_2 to C_0 ratio was found to be 5.2±1.9 (Table 2).

TABLE 2. Mean ± SD C0, C2 & c2/c0 in renal transplant

CsA	Mean (SD)
C2	1018.77 (278.969)
C0	194.13 (76.863)
C2/C0	5.674 (1.9530)

Pearson's correlation test depicted significant difference between C_2/C_0 and C_0 ($p<0.001$) and a direct correlation with C_2 (Table 3).

TABLE 3. Relative correlation of C_2 / C_0 with clinical parameters

	Ratio C_2/C_0	
	Pearson correlation	Sig. (2-tailed)
Ratio C_2/C_0	1	-
C_2 level ng/ml	-.118	.498
C_0 level ng/ml	-.567	.000
Hb%	.151	.387
SBP	-.136	.435
DBP	-.079	.654
Age (yrs)	-.030	.863
Gender	-.179	.304
Serum urea mg/dl	-.123	.481
Serum creatinine mg/dl	.008	.965
Morning dose	-.205	.238
TGs mg/dl	.051	.773
Weight (kgs)	.223	.198
Uric acid mg/dl	-.047	.78
Transplant period (yrs)	.092	.600

Analysis of effect of time since transplant on target C_2/C_0 revealed that ratio of 5.0±0.8 within six months or 5.3±0.6 within one year of transplant was not significantly different thereafter i.e. 5.7±2.4 after 6 months and 5.7±2.1 after one year (Figures 2 and 3).

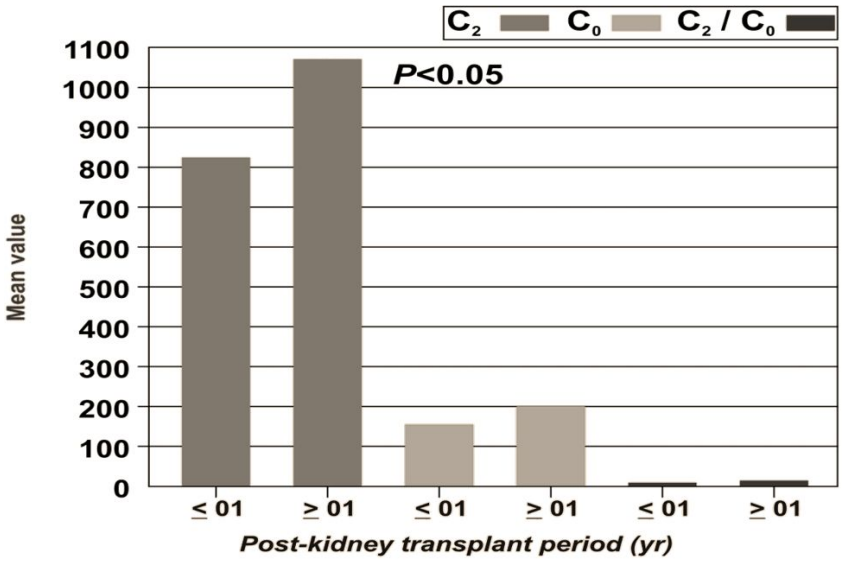


Fig. 2. Mean ± SD CsA exposure before and after 1 year of transplant

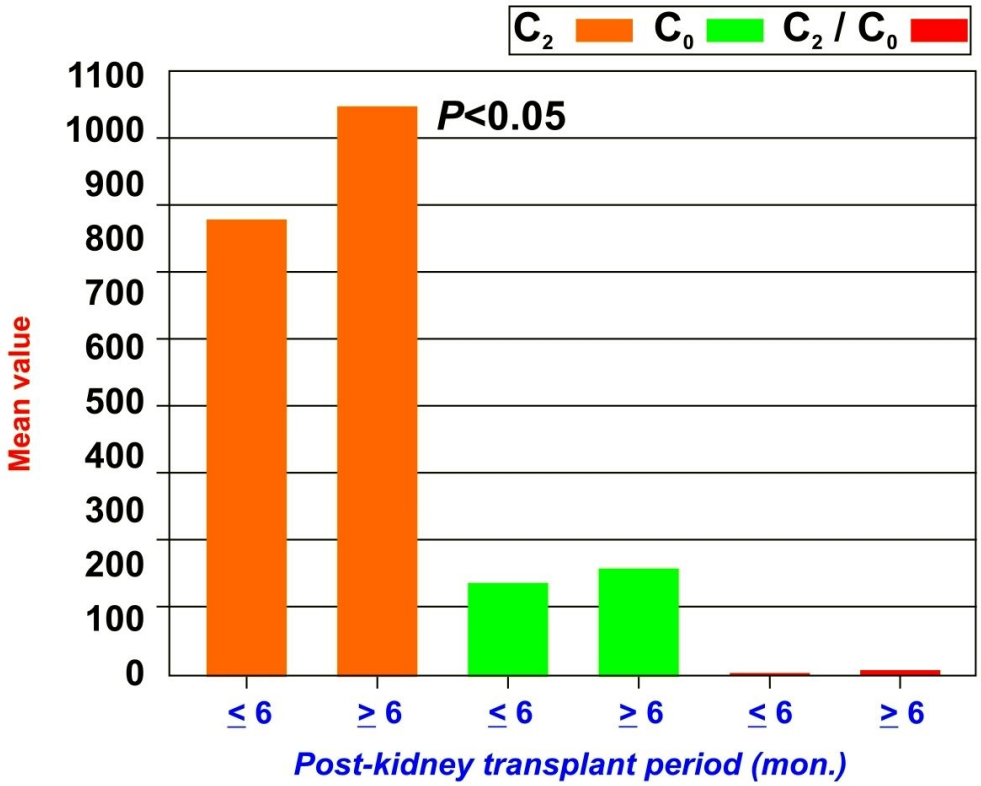


Fig. 3. Mean + SD CsA exposure before and after 6 months of transplant
All the patients were on triple therapy.

Discussion

Monitoring C_0 and C_2 Cyclosporine levels in a cohort of 35 kidney transplant patients with stable function but variable individual treatment factors was attempted to aim at devising metrics of graft exposure to CsA and setting an algorithm as a guideline that could help avoid complications of over and under exposure in transplant follow-up patients irrespective of significant differences in bioavailability due to food, circardian rhythm, unequal dose increments and other biological variations. Despite routine TDM of CsA for past two decades, the issues of monitoring remain yet to be resolved. Recognition of relatively poor relationship of C_0 to CsA exposure evolved the concept of area under blood concentration curve versus time especially for first 4 hours after dose (AUC_{0-4}) to have an insight into how well absorption takes place but remained poorly accepted in practice. Post dose peak (C_2) proved clinically more acceptable single sample alternative for AUC_{0-4} and came to be used as surrogate index of CsA absorption and exposure [12]. This argument is ultimately based on pharmacodynamic benefits expected of direct calcinurine inhibition in tissues that is maximally achievable in parallel to CsA concentration at around 2 hours after dose [13]. The necessity to have such algorithm was felt due to contradicting reports [14, 15] regarding predictive values of C_0 and C_2 as indicators of graft exposure to CsA. C_0 of 194.1±76.8 and C_2 of 1018.7±278.9ng/ml during maintenance treatment at steady state irrespective of age, gender, period of transplant, concomitant immunosuppressive therapy and factors affecting oral bioavailability were consistent with acceptable renal function. C_2 to C_0 ratio was found to be 5.2±1.9 (5.0 to 6.3). C_0 depicted significant difference with C_2/C_0 *($p<.001$)* as compared to C_2 which showed direct correlation. The safety profile including no evidence of ADR's could be better related to the ratio than any single parameter.

In conclusion, whether to use C_0, C_2 or C_2/C_0 as the monitoring tool for CsA will depend upon the transplant physician and surgeon but the laboratory approach will help to relate PK to PD patients outcomes with better safety margin. The study needs more samples in order to bring a real picture so that these two levels can become the part and parcel of the cyclosporine TDM programme. The limitations of Therapeutic Drug Monitoring in clinical practice are that it requires consistency in terms of drug administration and sampling. For example, meals may decrease the C_{max} and AUC of CNI (16, 17)

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