

# Laboratory Monitoring of Cyclosporine Pre-dose Concentration ( $C_0$ ) After Kidney Transplantation in Isfahan

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## Abstract

**Background:** Cyclosporine is the main immunosuppressive agent used in organ transplantation which leads to considerable improvement in graft survival. The large inter- and intra- patient variability in cyclosporine pharmacokinetics coupled with the agent's narrow therapeutic index and adverse effects necessitate therapeutic monitoring of cyclosporine blood levels.

**Objective:** The aim of this study was to determine the extent of variability following oral administration of cyclosporine after kidney transplantation, and provide guidelines for administration of cyclosporine in Isfahan/Iran

**Methods:** The results of 2163 cyclosporine pre-dose blood samples obtained from 647 kidney transplant recipients (208 females, 439 males) with a median age of 34 years (range 11-54 years) were studied. Concentration of cyclosporine in the whole blood was determined by a radioimmunoassay using monoclonal antibodies specific for the drug. Statistical analyses were performed using SPSS.

**Results:** The frequency distribution of  $C_0$  and daily oral dosage of cyclosporine exhibited wide interindividual variability. Cyclosporine oral dosage regimen ranged from 100 mg to 400 mg. Trough cyclosporine blood concentration ( $C_0$ ) ranged from 18 ug/l to 1400 ug/l. The results of cyclosporine whole blood levels in 56% were always below the suggested therapeutic range (less than 200 ug/l) and in 14% of the samples seemed to be associated with the occurrence of toxic side effects. There were no significant differences in the median trough levels of kidney recipients according to gender ( $p = 0.36$ ).

**Conclusion:** For long-term management of kidney transplant recipients and in order to further optimize the use of cyclosporine, it is essential to standardize laboratory monitoring and clinical investigation of this agent.

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**Keywords** • Laboratories • kidney transplantation • cyclosporine

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## Introduction

**A** characteristic feature of all studies performed using cyclosporine pre-dose concentrations ( $C_0$ ), has been the emphasis on drug monitoring.<sup>1,2</sup> Disparities in blood levels are the result of pronounced differences in the pharmacokinetic parameters, such as clearance and bioavailability. These inter- and intra- individual variations may influence the immune response and susceptibility to drug toxicity.<sup>3,4</sup> The absorption of cyclosporine is slow and highly variable after oral administration and appears to be influenced by a variety of factors such as, the time after transplant, presence of food and intestinal dysfunction.<sup>5,6</sup> Distribution is affected by the lipoprotein concentration in plasma and by the haematocrit.<sup>7,8</sup> Heterogeneity in gut CYP450A gene expression also explains some of the wide variability in cyclosporine kinetics. In addition, drugs that induce or inhibit CYP450A influence the CYP450A -dependent metabolism.<sup>9</sup> Demographic factors such as age, gender and race may also contribute to the variability of cyclosporine pharmacokinetics.<sup>10</sup> Among renal allograft recipients, there is a considerable variability in cyclosporine trough levels.<sup>4</sup> Cyclosporine is extensively metabolized and hepatic metabolism is a primary pathway of cyclosporine elimination.<sup>9</sup> The total body clearance of cyclosporine is markedly variable in patients and is influenced by the nature of transplant, patient's age, any associated disease state and concurrent drug therapy.<sup>7,10</sup> Renal side-effects of cyclosporine are dose-related, but the influence of the dosage regimen has not been thoroughly investigated.<sup>11</sup> Therapeutic drug monitoring of this agent involves the use of blood concentration measurements of the drug to individualize dosage regimens based on pharmacokinetic principles.<sup>1</sup> The traditional method of optimizing a cyclosporine dose regimen in a patient is by titrating the pre-dose blood concentration of cyclosporine (trough level) to a designated range that is considered therapeutic and non-toxic.<sup>3,12</sup> Due to the narrow therapeutic index, optimal dosing of cyclosporine in the individual patient can only be achieved by the inclusion of therapeutic drug monitoring as an essential component of the patient's regimen, and on a regular basis after the initiation of therapy.<sup>3,4,12</sup> To achieve good survival rates, keep morbidity to an acceptable level and to provide guidelines for therapy with cyclosporine,<sup>4,12</sup> the management of immunosuppression should minimize the incidence of graft rejection (acute or chronic) and maintain drug levels within therapeutic ranges.<sup>13,14</sup> Therefore, the aim of this work was to study, inter-individual variability in cyclosporine pre-dose concentrations ( $C_0$ ) and the relevance of ma-

**Table 1:** Cyclosporin trough concentration:  $C_0$  (ug/l)

	Total group (n=2163)	Female (547)	Male (1616)
Mean*	240	234.3	245.7
Median	182	198	181
SD	411.1	398	402.2
Min	18	18	21
Max	1400	1350	1400

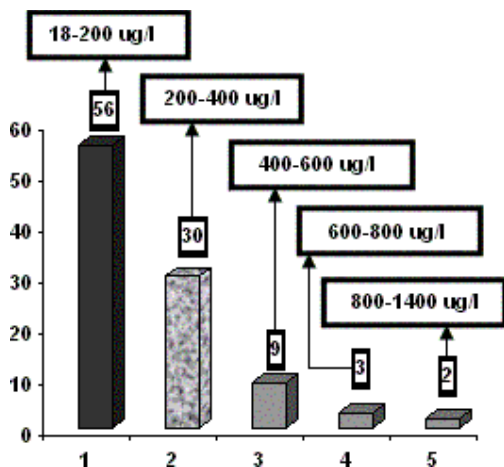
\*P value = 0.36

nipulating the dose of this agent in response to drug levels.

## Patients and Methods

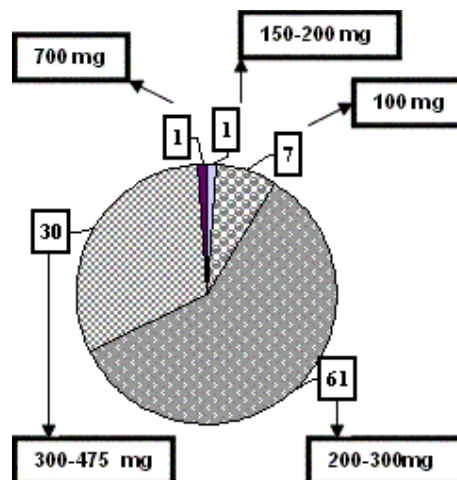
Patients (208 female, 439 male) with a median age of 34 years (mean 34.1, range 11-54 years) receiving primary kidney grafts for end stage renal disease (ESRD), at Kidney Transplant Center, Isfahan University of Medical Sciences, were studied. All patients received cyclosporine orally twice a day (7.9 mg/kg/day; range 3-11 mg/kg/day)<sup>13,15</sup> and approximately 2163 whole blood pre-dose samples (547 samples belonged to females & 1616 samples belonged to males) were analyzed. The blood samples were extracted with methanol and after centrifugation, the supernatant of unknown samples and standards were incubated with <sup>125</sup>I-labelled cyclosporine in monoclonal specific anti-cyclosporine coated tubes. The bound radioactivity was measured using a  $\gamma$ -scintillation counter Multi-gama. The calibration range of the method was 10-1380 ug/l and the sensitivity about 10 ug/l. Additional information including study date, date of transplant, and demographic data were recorded in MS-Excel. Statistical analyses were performed using SPSS program version 6. The Shapiro-Wilks tests were used to confirm normal distribution of data. When the data were not normally distributed and equal variances among groups could not be assumed, non-parametric Mann-Whitney tests were used. Correlation between variables was determined by Spearman Rank order correlation analysis. Significant differences were accorded at *P* values <0.05.

## Results



**Figure 1:** Inter-individual variability (%) in trough levels ( $C_0$ ,  $n = 2163$ ) of cyclosporin after kidney transplantation in Isfahan/Iran. This fig shows that 56% of the results were less than 200  $\mu\text{g/l}$ , which, seems below the suggested therapeutic range.

This study demonstrated that the distribution of pre-dose blood levels of cyclosporine ( $C_0$ ) followed different groups of populations. The Shapiro-Wilks test confirmed that the data were not normally distributed. As shown in Table 1, with a mode of 100  $\mu\text{g/l}$ , the median cyclosporine whole blood trough concentration after kidney transplantation was 182  $\mu\text{g/l}$  and the mean value with one standard deviation of 411.1  $\mu\text{g/l}$  was 240  $\mu\text{g/l}$  ( $n = 2163$ ). The lowest level of cyclosporine observed in this study was 18  $\mu\text{g/l}$  and the highest level, which might be related to toxicity, was 1400  $\mu\text{g/l}$ . However, the administration of cyclosporine was associated with marked variability and large variation in  $C_0$ , but both the effectiveness and toxicity of cyclosporine appeared to be related to the level of drug exposure within study population. The higher values of median  $C_0$  in kidney transplant recipients most likely reflected existing saturation of the compartments into which cyclosporine is distributed. The higher results could therefore be considered as reflecting a reduced apparent volume of distribution for the drug. However, decreased absorption is also possible. As the distribution of  $C_0$  follows different groups of population, the samples were stratified according to blood levels of cyclosporine, as can be seen in Figure 1. The results of cyclosporine trough blood levels in 56%, were always (less than 200  $\mu\text{g/l}$ ) below the suggested therapeutic range. Therefore, low cyclosporine concentrations in blood, due to poor drug absorption or rapid elimination, should be recognized and adjusted early as an important part of the care of transplant recipients.



**Figure 2:** Inter-patient variability (%) in doses (mg) of cyclosporine after kidney transplantation in Isfahan/Iran. Low doses of cyclosporine might increase the possibility of graft rejection due to insufficient therapeutic blood level.

In 30% of the samples, cyclosporine blood levels were more than 200  $\mu\text{g/l}$  and less than 400  $\mu\text{g/l}$ . In 12%, the results of cyclosporine blood levels were more than 400  $\mu\text{g/l}$  and less than 800  $\mu\text{g/l}$ . In a total of 14% levels of cyclosporine ( $C_0 > 400 \mu\text{g/l}$ ) seemed to be on the toxic side, which might be associated with the occurrence of infectious episodes, nephro-,hepato- and neurotoxic side effects. Cyclosporine blood levels correlated poorly with dose ( $r = 0.031$ ,  $p = 0.23$ ). Analysis of trough concentrations by gender showed no significant difference in trough levels of cyclosporine ( $p = 0.36$ ).

As can be seen in Figure 2, a further analysis of dosage regimens showed that daily oral cyclosporine dose varied among different groups. The mean dose was 298 mg daily (range: 100-700 mg) ( $SE=6.21$ ,  $mode=250$ ,  $median=300$ ). As shown in Figure 2, 7% of recipients were received 100 mg of cyclosporine daily, 61% of recipients received 200-300 mg and 30 % received 300-475 mg of cyclosporine, which seems to be lower than recommended therapeutic dosage of this drug.

## Discussion

The review of literature shows that, for a highly successful treatment after kidney transplantation, monitoring of cyclosporine concentration is essential.<sup>3,4,12</sup> Immunosuppressive therapy using cyclosporine is a narrow path between the risk of rejection by underimmunosuppression and toxic organ damage by overdose.<sup>14,16,17</sup> The present study was organized to correspond closely to inter-

individual variability in trough levels of cyclosporine after kidney transplantation in Isfahan/Iran. Because of its wide variability (18 to 1400 ug/l), cyclosporine requires individualization of doses to tailor immunosuppression to the specific patient.<sup>12,14</sup>

Wide inter-individual variability might be related to the excretion (and therefore persistence) of different metabolites and the amount produced among individual recipients.<sup>18,19</sup> Differences in absorption and clearance of cyclosporine observed in this study might be explained by pharmacokinetic variability and result both from genetic determinants and clinical status (e.g. hepatic and extra-hepatic organ function). In addition, identification of biochemical indicators of clinical status with which these wide variability are associated might facilitate clinical management, complementing therapeutic drug monitoring results.<sup>12</sup> These points merit further investigations in relation to clinical outcome.

Since graft rejection (acute or chronic) is an important cause of long-term allograft loss, the large inter-individual variability observed in this study may have a significant impact.<sup>14,16,20,21</sup> Previous studies showed that a cyclosporine concentration exceeding approximately 182 ug/l is essential to prevent graft rejection<sup>14,16</sup> and cyclosporine levels of 200–400 ug/l inhibit calcineurin phosphatase activity in humans by about 50%.<sup>22</sup> It seems that values of less than 200 ug/l, observed in 56% of cyclosporine blood samples, could not achieve complete calcineurin inhibition.<sup>22</sup>

The findings of this work is in accordance with earlier publications hence it is recommended that optimal dosing in the individual patient be achieved by the inclusion of therapeutic drug monitoring as an essential component of the patient's long-term management plan.<sup>12,19</sup> Therefore, to avoid the risk of organ rejection through underdosage,<sup>14,16,19</sup> as observed in 56% of the samples and also toxic organ damage through over-dosage of cyclosporine<sup>14</sup>, which were seen in 14% of the samples, dosage of cyclosporine should be calculated on the basis of daily trough level measurements.<sup>12,19,23</sup>

Finally for transformation of renal transplantation from an experimental procedure to a highly successful treatment that is universally recognized as the optimal clinical therapy for end-stage renal disease (ESRD), in addition to the potential of laboratory monitoring of cyclosporine pre-dose concentration, dosage adjustment should merit further clarification in relation to clinical outcome.

## References

1 Kahan BD: The evolution of therapeutic immunosuppression and the potential impact of drug

- concentration monitoring. *Therapeutic Drug Monitoring*. 1995;**17(6)**: 560-3.
- 2 Ptachcinski RJ, Venkataramanan R, Rosenthal JT, et al: Cyclosporine pharmacokinetics in renal transplant recipients. *Clin Pharmacol Ther*.1985;**38(3)**:296-303.
- 3 Tsunoda SM, Aweeka FT: The use of therapeutic drug monitoring to optimise immunosuppressive therapy. *Clin Pharmacokinet* 1996;**30(2)**: 107-40.
- 4 Andany MA, Kasiske BL: Care of the kidney transplant recipient. Vigilant monitoring creates the best outcome. *Postgrad Med* 2002; **112(3)**:93-6, 101-2, 105-8 passim.
- 5 Kahan BD, Welsh M, Schoenberg L, et al: Variable absorption of cyclosporine. *Transplantation* 1996; **62(5)**: 599-606.
- 6 Trull AK, Tan KK, Tan L, et al: Absorption of cyclosporine from conventional and new microemulsion oral formulations in liver transplant recipients with external bile diversion. *Br J Clin Pharmacol* 1995; **39(6)**: 627-631.
- 7 Fahr A: Cyclosporine clinical pharmacokinetics. *Clin Pharmacokinet*. 1993; **24(6)**:472-95.
- 8 Akhlaghi F, Trull AK: Distribution of cyclosporine in organ transplant recipients. *Clin Pharmacokinet*. 2002; **41(9)**:615-37.
- 9 Sewing KF, Christians U, Kohlhaw K, et al: Biologic activity of cyclosporine metabolites. *Transplant Proc* 1990; **22(3)**: 1129–34.
- 10 Christians U, Bleck JS, Lampen A, et al: Are cytochrome P450 3A enzymes in the small intestine responsible for different cyclosporine metabolite patterns in stable male and female renal allograft recipients after co-administration of renal allograft recipients after co-administration of diltiazem? *Transplant Proc* 1996; **28(4)**: 2159-61.
- 11 Canafax DM: Minimizing cyclosporine concentration variability to optimise transplant outcome. *Clin Transplant* 1995; **9(1)**: 1–13.
- 12 Tolou-Ghamari Z: Monitoring tacrolimus in liver transplant recipients: a consideration of alternative techniques and the influence of clinical status. PhD thesis 1999; University of London.
- 13 Inoue S, Beck Y, Nagao T, Uchida H: Early fluctuation in cyclosporine A trough levels affects long-term outcome of kidney transplants. *Transplant Proc* 1994;**26(5)**:2571-3.
- 14 Min DI, Perry PJ, Chen HY, et al: Cyclosporine trough concentrations in predicting allograft rejection and renal toxicity up to 12 months after renal transplantation. *Pharmacotherapy* 1998; **18(2)**: 282-7.
- 15 Bock HA, Kamber V, Brunner FP, et al: Weight-independent dosing of cyclosporine--an alternative to the "mg/kg" doctrine. *Transplantation* 1994;**57(10)**:1484-9.

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- 16 Gjertson DW: Impact of delayed graft function and acute rejection on graft survival. *Transplant Proc* 2002;**34(6)**:2432.
- 17 Ohlman S, Lindholm A, Hagglund H, et al: On the intraindividual variability and chronobiology of cyclosporine pharmacokinetics in renal transplantation. *Eur J Clin Pharmacol* 1993;**44(3)**:265-9.
- 18 Lindholm A, Kahan BD: Influence of cyclosporine pharmacokinetics, trough concentration and AUC monitoring on outcome of kidney transplantation. *Clin Pharmacol Ther* 1993; **54(2)**: 205-18.
- 19 Tolou\_Ghamari Z, Wendon J, Tredger JM: *In-vitro* pentamer formation as a biomarker of tacrolimus-related immunosuppressive activity after liver transplantation. *Clin Chem Lab Med* 2000;**38(11)**: 1209-11.
- 20 Salomon D, Brunson M, Vansickler J, et al: A retrospective analysis of late renal graft function: correlation with mean cyclosporine levels and lack of evidence for chronic cyclosporine toxicity. *Transplant Proc* 1991;**23(1pt2)**:1018-9.
- 21 Tan KK, Trull AK, Hue KL, et al: Pharmacokinetics of cyclosporine in heart and lung transplantation candidates and recipients with cystic fibrosis and Eisenmeenger's syndrome. *Clin Pharmacol Ther* 1993;**53(5)**: 544-54.
- 22 Batiuk TD, Pazderka F, Enns J, et al: Cyclosporine inhibition of calcineurin activity in human leukocytes in vivo is rapidly reversible. *J Clin Invest* 1995;**96(3)**: 1254-60.
- 23 Lindholm A: Factors influencing the pharmacokinetics of cyclosporine in man. *Ther Drug Monit* 1991;**13(6)**: 465-77.