

Association Between CYP3A5 Genetic Polymorphisms with Tacrolimus Dose Requirement and Allograft Outcomes in Iranian Kidney Transplant Recipients

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Keywords. CYP3A, kidney transplantation, polymorphism, tacrolimus

Introduction. Tacrolimus is the cornerstone of immunosuppressive therapy in organ transplantation with variable inter-individual pharmacokinetics. This study assessed the relationship between CYP3A5/3A4 polymorphisms and tacrolimus dose requirement as well as 6-month transplant outcomes in Iranian kidney transplant recipients.

Methods. In this prospective study, 110 adult kidney transplant recipients treated with tacrolimus were genotyped for the presence of common SNPs: rs776746: A > G (CYP3A5*3). Patients who carried at least one CYP3A5*1 allele were known as CYP3A5 expressers while those who were CYP3A5*3/*3 homozygotes were classified as CYP3A5 non-expressers.

Results. The daily tacrolimus dose was significantly higher and tacrolimus dose adjusted trough levels (C/D ratio) was significantly lower in CYP3A5 expressers compared with non-expressers ($P < .05$). Although the incidence of clinically suggested acute allograft rejection was significantly higher (OR = 0.365 [95% CI: 0.14 - 0.93]; $P < .05$) and median time to first acute rejection was sooner among CYP3A5 expressers compared with non-expressers (12.17 vs. 26.83 days, $P < .05$); however, estimated glomerular filtration rate, incidence of biopsy proven acute rejection and delayed graft function and 6-month patients' and grafts' survival did not differ between the two groups.

Conclusion. CYP3A5 genetic polymorphism is significantly associated with required tacrolimus dose. After achieving desired tacrolimus blood level, although some transplant outcomes such as the incidence of clinically suggested acute rejection and time to first rejection were different between CYP3A5 expressers and non-expressers, however, other clinical outcomes did not differ between groups. Therefore, it is not the time to routinely assess kidney transplant recipients for CYP3A5 genetic polymorphism before transplantation.

IJKD 2019;13:404-13
www.ijkd.org

INTRODUCTION

Tacrolimus (TAC), a calcineurin inhibitor, is the cornerstone of immunosuppressive therapy in many solid organ transplant centers.^{1,2} Because of

its highly variable pharmacokinetics and a narrow therapeutic index, therapeutic drug monitoring and concentration controlled dosing of TAC have been routinely applied for reducing the risk of

nephrotoxicity and allograft rejection.^{3,4} Some clinical and genetic factors are responsible for variable pharmacokinetic properties of TAC.^{5,6} A member of cytochrome P450 3A (CYP3A) subfamily, CYP3A5; is the major enzyme that is responsible for the metabolism of tacrolimus.⁷ Single nucleotide polymorphisms (SNPs) in intron 3 of the CYP3A5 gene have been found to partly explain the inter-individual variability in TAC metabolism and clearance.⁸ The CYP3A5*3 allele (G at position 6986) produces a cryptic splice site and encodes an abnormal mRNA splicing with a premature stop codon, while CYP3A5*1 allele (A at position 6989) produces a normal mRNA and higher expression of this enzyme in the liver and intestine.^{9,10} Based on previous studies, kidney allograft recipients possessing at least one CYP3A5*1 allele (known as CYP3A5 expressers) require 1.5 to 2 folds higher TAC doses to achieve therapeutic blood concentrations¹¹ and 2-fold lower TAC concentrations/dose ratio compared to CYP3A5 non-expressers (CYP3A5*3/*3 homozygotes).¹² The impacts of CYP3A5 polymorphism on kidney allograft outcomes such as acute rejection episodes and level of kidney function are controversial.¹³⁻¹⁵ The relationship between CYP3A5/3A4 polymorphisms and TAC dose requirement has not been studied in Iranian kidney transplant patients. We have assessed the CYP3A5/3A4 polymorphisms and TAC dose requirement as well as 6-month graft outcomes in Iranian kidney transplant recipients.

MATERIALS AND METHODS

Patients and Immunosuppressive Protocols

This prospective, cross-sectional, multicenter study was conducted from 6 November 2016 to 7 August 2018 at Kidney Transplant Wards of Imam Khomeini Hospital Complex and Milad Hospital, Tehran, Iran.

Adult kidney transplant recipients from deceased or living donors who received thymoglobulin induction therapy and maintenance immunosuppressive regimen containing tacrolimus, mycophenolate (mofetil or sodium), and prednisolone were included. Thymoglobulin induction was started at a daily dose of 1 mg/kg one hour before transplantation and continued daily to a cumulative dose of 3 to 4 mg/kg. According to these centers' protocols, all patients

received intravenous methylprednisolone that was administered at doses of 500, 250, and 125 mg; respectively for day 1, 2, and 3 of transplantation and followed by 1 mg/kg/d of oral prednisolone with rapid taper down to 5 mg/d after one month of kidney transplantation. Tacrolimus, mycophenolate (mofetil or sodium), and prednisolone were maintenance immunosuppressive drugs in these centers. All patients in this study received tacrolimus (Prograf[®], Astellas, Netherland) from the day after transplantation and were advised to receive their TAC doses 2 hours after breakfast and dinner at 9:00 am and 9:00 pm. In these centers, the target TAC whole blood trough concentration was 8–10 ng/mL within the first three months after kidney transplantation. All patients received ganciclovir/valganciclovir, co-trimoxazole, and clotrimazole as their routine prophylaxis against viral infections, *Pneumocystis jiroveci* pneumonia, and fungal infections; respectively for defined period based on the centers' protocols. Consumption of foods and fruits that have considerable interactions with TAC metabolism (*e.g.* grapefruit, high amount of bitter orange, and Earl Gray tea) and any herbal agents (especially St. John's Wort) were routinely prohibited in kidney transplant recipients in these centers. Patients with multi-organ transplantations, patients who received any drug with potential to induce (such as phenytoin, phenobarbital, carbamazepine, rifampin) or inhibit (such as macrolide antibiotics, azole antifungal agents, calcium channel blockers, protease inhibitors) TAC metabolism, patients younger than 14 years old, who switched to other immunosuppressive regimen during study follow-up or in the case of loss to follow-up were excluded from the study.

Data Collection

Demographic, clinical, and laboratory data of the recipients and donors were collected from the medical reports. Delayed graft function was defined as the need for dialysis within the first week after transplant surgery. CMV and polyoma (BK) virus infections were defined as blood count of 10³ IU/mL and 10⁴ copies/mL using polymerase chain reactions, respectively. Protocol allograft biopsy was not done routinely in these centers. Indication biopsies were performed based on physicians' decision and patients' satisfaction. All kidney biopsy specimens from both centers

were evaluated according to Banff criteria¹⁶ by one nephropathologist.

Tacrolimus blood concentrations were assessed using chemiluminescence micro particle immunoassay (Abbott, i2000, USA) on samples taken 30 minutes before morning TAC doses at steady state (*i.e.* at least 3 days after tacrolimus initiation or dosage changes). TAC metabolism rate was determined by dividing the whole blood trough concentration of the drug (C) (ng/mL) by the corresponding daily TAC dose (D) (mg/kg). The C/D ratio was calculated at the time of hospital discharge, months 1, 3, and 6 after transplantation using mean TAC daily dose and trough concentrations during past days/months.

Estimated GFR (eGFR) was calculated using 4-variables modification of diet in renal disease (MDRD) study equation at the time of hospital discharge, and months 1, 3, and 6 after transplantation. Patients who lost their graft during the study period remained in data analysis by considering eGFR of 0 mL/min/ 1.73m² for them.¹⁷

Genotyping

Genomic DNAs were extracted from whole-blood samples in EDTA tubes using the High Pure PCR Template Preparation Kit (Roche Applied Science, Germany). Genotyping for the CYP3A5 (6986A > G; rs776746) single nucleotide polymorphism was performed using TaqMan® allelic discrimination assay (Applied Biosystems, Life Technologies, USA) on an StepOnePlus® Real-Time PCR (Applied Biosystems, USA) according to the manufacturer's instructions. Briefly, the PCR cycle consisted of an initial step of 30 seconds at 60 °C, followed by a denaturation step at 95 °C for 15 minute and 40 cycles with 95 °C for 15 seconds and 60 °C for 90 seconds. The volume for each reaction was 10 µL, consisting of 5 µL RealQ Plus 2x Master Mix for Probe, High ROX (Ampliqon, Denmark), 0.5 µL of TaqMan® Genotyping Assays (20X), containing the GBM probes and primers with a concentration of 18 µM for each primer and 4 µM for the probe, and 2 µL of genomic DNA. Patients carried at least one CYP3A5*1 allele were known as CYP3A5 expressers while those who were CYP3A5*3/*3 homozygotes were considered as CYP3A5 non-expressers

Statistical Analysis

Data was analyzed using SPSS (SPSS Inc.,

Chicago, IL, USA) version 22. Normal distributions of quantitative variables were assessed using Kolmogorov-Smirnov test. Normal and non-normal distributed variables are presented as mean ± SD and median (minimum-maximum), respectively. Categorical variables were compared between different CYP3A5 genotype groups using Chi-square or Fisher exact tests. Tacrolimus daily dose, trough blood levels and C/D ratios and eGFR were compared between CYP3A5 expressers and non-expressers and between males and females using independent-sample t-test or Mann-Whitney U test, which appropriate. Correlations between quantitative variables were assessed using Pearson or Spearman tests which appropriate. ANCOVA was used to assess association between mean C/D ratio during 6-months post transplantation and some independent qualitative and quantitative variables.

Ethics

The study protocol was approved by Local Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.REC.1394.1310). All patients signed informed consent forms.

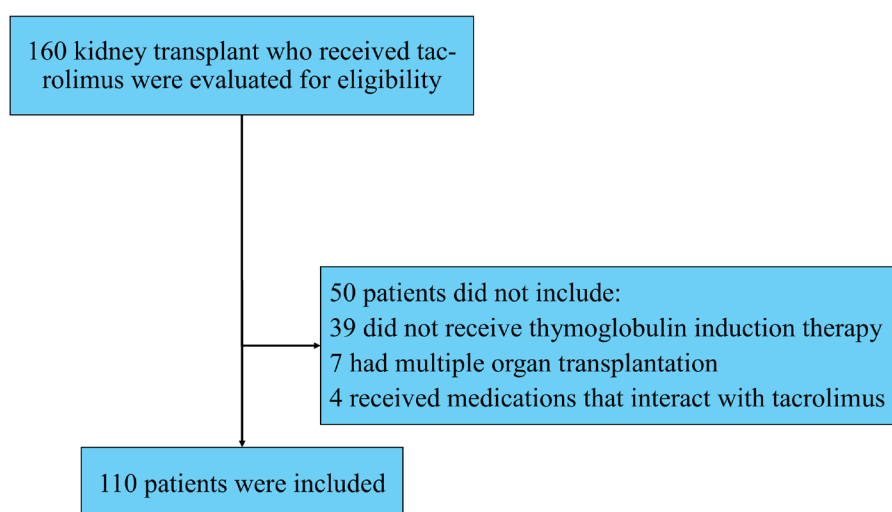
RESULTS

Characteristics of Study Population

A total of 110 adult kidney transplant recipients were included in the present study (Figure). The CYP3A5 allele frequencies of *1/*1, *1/*3 and *3/*3 genotypes in these allograft recipients were 1 (0.9%), 23 (20.9%), and 86 (78.2%); respectively. The clinical and laboratory characteristics of the kidney transplant recipients and donors did not differ between CYP3A5 expressers and non-expressers (Table 1).

Effect of CYP3A5 Genetic Polymorphism on Tacrolimus Blood Levels, Daily Dose and C/D Ratio

To maintain tacrolimus blood concentrations within desired range, the daily tacrolimus dosage was significantly higher in CYP3A5*1 allele carriers compared with that in CYP3A5*3/*3 carriers during the study period ($P < .05$, Table 2) and consequently, tacrolimus C/D ratio was significantly lower among CYP3A5 expresser patients compared to CYP3A5 non-expressers, ($P < .05$, Table 2).



A total of 110 adult kidney transplant recipients were included in the present study.

Table 1. Comparison of the Clinical Characteristics of the Study Population Between CYP3A5 Expressers and Non-expressers

Characteristics	CYP3A5 Genotypes		P
	*1/*1+*1/*3	*3/*3	
Age, y	46.17 ± 12.19	44.64 ± 13.31	> .05
Gender, n (%)			
Male	15 (62.5)	64 (74.4)	> .05
Female	9 (37.5)	22 (25.6)	
Weight, kg	66.04 ± 15.19	70.71 ± 14.51	> .05
BMI, kg/m ²	22.93 ± 3.81	24.93 ± 4.65	> .05
Duration of Dialysis, mo	26.04 ± 23.88	23.55 ± 23.56	> .05
Panel Reactive Antibody, %	0 (0 - 4)	0 (0 - 4)	> .05
Donor Type, n (%)			
Deceased	21 (87.50)	75 (87.20)	> .05
Living	3 (12.5)	11 (12.79)	
Gender of Donor, n (%)			
Male	16 (66.67)	52 (61.17)	> .05
Female	8 (33.34)	34 (39.53)	
Age of Donor, y	35.57 ± 13.35	36.33 ± 13.53	> .05
Weight of Donor, kg	72.00 ± 13.39	70.49 ± 11.56	> .05
BMI of Donor, kg/m ²	25.38 ± 4.25	24.74 ± 3.07	> .05
Last SCr of Donor, mg/dL	1.24 ± 0.41	1.23 ± 0.37	> .05

The values are presented as mean ± SD and n (%) as indicated. BMI, body mass index; SCr, serum creatinine concentration

Effect of CYP3A5 Genetic Polymorphism on Graft Outcomes

Incidence of clinically suggested acute rejection was significantly higher among recipients carrying at least one CYP3A5*1 allele compared to patients with CYP3A5*3/*3 genotype (50% *vs.* 26.7%, $P < .05$; Table 3). Only 5 patients in CYP3A5 expresser group and 9 patients in CYP3A5 non-expresser group underwent caused allograft biopsy, all showed acute rejection (Table 3) without evidence of tacrolimus nephrotoxicity.

Acute rejection episodes occurred earlier in CYP3A5 expressers compared to CYP3A5 non-expressers ($P < .05$, Table 3). In addition, CYP3A5 expressers had longer length of hospital stay ($P < .05$, Table 3). Incidence of delayed graft function, level of kidney function (eGFR), patients' survival and allograft loss over the six months post transplantation did not differ between CYP3A5 expressers and non-expressers (Table 3). The rates of CMV and BK virus infections were not significantly different between CYP3A5 expressers

Table 2. Comparing Tacrolimus Blood Levels, Daily Dose and C/D Ratio Between CYP3A5 Expressers and Non-expressers

CYP3A5	Mean (SD)	Median (min-max)	P
C, ng/mL			
At Hospital Discharge			
*1/*1 + *1/*3	9.19 (3.06)	8.80 (4.80 - 15.20)	> .05
*3/*3	9.69 (3.48)	9.20 (2.40 - 23.00)	
Month 1			
*1/*1 + *1/*3	9.24 (3.02)	8.31 (3.95 - 14.77)	> .05
*3/*3	10.33 (3.51)	10.04 (2.40 - 27.56)	
Month 3			
*1/*1 + *1/*3	7.04 (2.31)	6.47 (4.70 - 15.10)	< .001
*3/*3	10.22 (4.51)	9.20 (3.60 - 29.35)	
Month 6			
*1/*1 + *1/*3	7.67 (1.67)	7.45 (5.60 - 12.60)	> .05
*3/*3	8.26 (1.96)	8.00 (4.50 - 13.70)	
Dose, mg/kg/d			
At Hospital Discharge			
*1/*1 + *1/*3	0.09 (0.03)	0.09 (0.06 - 0.16)	< .05
*3/*3	0.08 (0.02)	0.08 (0.03 - 0.17)	
Month 1			
*1/*1 + *1/*3	0.10 (0.04)	0.09 (0.06 - 0.24)	< .001
*3/*3	0.08 (0.02)	0.08 (0.04 - 0.13)	
Month 3			
*1/*1 + *1/*3	0.10 (0.03)	0.09 (0.05 - 0.18)	< .001
*3/*3	0.07 (0.02)	0.07 (0.02 - 0.15)	
Month 6			
*1/*1 + *1/*3	0.09 (0.03)	0.08 (0.05 - 0.19)	< .001
*3/*3	0.06 (0.02)	0.06 (0.02 - 0.11)	
C/D ratio, ng/mL per mg/kg/d			
At Hospital Discharge			
*1/*1 + *1/*3	107.47 (53.09)	91.09 (45.04 - 220.57)	< .05
*3/*3	134.32 (64.32)	116.40 (23.60 - 368.29)	
Month 1			
*1/*1 + *1/*3	99.70 (46.49)	97.78 (21.33 - 217.60)	< .05
*3/*3	148.29 (64.48)	143.36 (23.60 - 363.07)	
Month 3			
*1/*1 + *1/*3	78.05 (37.59)	69.25 (29.52 - 200.58)	< .001
*3/*3	164.74 (88.17)	142.63 (24.69 - 497.93)	
Month 6			
*1/*1 + *1/*3	92.17 (38.52)	89.88 (31.85 - 160.02)	< .001
*3/*3	168.77 (92.40)	151.11 (61.20 - 534.93)	

C, tacrolimus trough blood concentration; D, tacrolimus daily dose; SD, standard deviation

and non-expressers ($P > .05$).

During the study period TAC daily dose, blood levels and C/D ratios were compared between male and female transplant recipients. For achieving similar TAC blood levels, female patients needed significantly higher daily TAC dose ($P < .05$, Table 4) and therefore had significantly lower C/D ratio compared with males ($P < .05$, Table 4). eGFR was not significantly different between male and female recipients during the 6 months follow-up (Table 4).

Among other demographic characteristics,

recipient's age showed significant correlation with mean C/D ratio during 6 months post transplantation ($r = 0.265$, $P < .05$). The univariate analysis (ANCOVA) confirmed the significant association of the three selected independent variables including recipients' gender, age, and CYP3A5 genotype; with tacrolimus C/D ratio during six months post-transplantation ($P < .05$, Table 5).

DISCUSSION

To our knowledge, this is the first pharmacogenetic

Table 3. Clinical Outcomes During 6 Months Post-transplantation CYP3A5 Expressers and Non-expressers

Parameters	CYP3A5		P
	*1/*1 + *1/*3	*3/*3	
DGF, n (%)	7 (29.2)	33 (38.4)	> .05
Acute Rejection, n (%)	12 (50.0)	23 (26.7)	< .05
Total Number of Acute Rejection	0 (0 - 3)	0 (0 - 3)	> .05
BPAR, n (%)	5 (20.8)	9 (10.5)	> .05
Time to First Acute Rejection (days)	12.17 (8.89)	26.83 (23.53)	< .05
	10.50 (2 - 37)	15 (3 - 74)	
Patient Survival, n (%)	23 (95.8)	82 (95.3)	> .05
Graft Loss, n (%)	4 (16.7)	6 (7.0)	> .05
LOS (days)	24.04 (15.75)	19.66 (11.87)	> .05
	18.50 (10.00 - 85.00)	15.00 (7.00 - 66.00)	
eGFR, mL/min/1.73m ²			
At Hospital Discharge	39.94 (12.40)	44.82 (15.90)	> .05
	39.86 (0 - 56.34)	43.45 (0 - 82.20)	
Month 1	43.70 (13.47)	47.46 (16.94)	> .05
	44.05 (12.81 - 67.83)	45.48 (8.21 - 87.97)	
Month 3	50.61 (22.54)	54.52 (18.81)	> .05
	54.64 (0 - 91.46)	57.22 (0 - 79.71)	
Month 6	48.57 (25.67)	55.68 (23.61)	> .05
	56.55 (0 - 79.71)	58.32 (0 - 99.85)	

The values are presented as mean \pm SD, [median (minimummaximum)] or n (%) as indicated. DGF, delayed graft function; BPAR, biopsyproven acute rejection; LOS: length of stay

study to evaluate the influence of CYP3A5 polymorphism on tacrolimus dose requirement and allograft outcomes in Iranian kidney transplant recipients throughout six months post transplantation.

There is a study on CYP3A5 genotypes among Iranian liver transplant recipients who took tacrolimus.¹⁸ One major difference between kidney and liver transplantation is that in liver transplant recipients there is a shift from the CYP450 of the recipient's native liver to that of the allograft. Thus, there are two different CYP3A5 systems at work in liver transplant recipients that make it hard to correlate tacrolimus dose requirement and liver transplant outcomes to only recipient's or donor's polymorphism.¹⁹ Another study on Iranian population evaluated the association between cyclosporine concentration and genetic polymorphisms of CYP3A5 during the early stage after renal transplantation.²⁰ Since the effect of CYP3A5 polymorphism on CNI clearance is drug specific and it has been suggested that CYP3A5 has lesser contribution on cyclosporine metabolism compared with tacrolimus metabolism,²¹ therefore; it is not easy to generalize pharmacogenetic studies on cyclosporine to tacrolimus in any specific solid organ transplant type.

The allele frequencies of CYP3A5*1 and *3 in our studied population were similar to those reported among Caucasians and Iranian population.^{11,18} Our results showed that carriers of at least one active allele (CYP3A5*1) needed significantly higher daily doses of tacrolimus compared to patients who were homozygous for CYP3A5*3 (CYP3A5 non-expressers) at the time of hospital discharge, months 3, and 6 after transplantation. These results support the fact that carriers of CYP3A5*1 allele exhibit high levels of CYP3A5 expression and enzymatic activity,^{9,22,23} leading to need for higher daily doses to obtain therapeutic trough levels of tacrolimus. The parallel, inverse results were achieved when we considered the effect of CYP3A5 genetic polymorphism on tacrolimus concentration/dose ratio; patients with CYP3A5*3/*3 genotype had significantly higher C/D ratios compared with those who carried at least one allele of CYP3A5*1. This finding is compatible with the result of a meta-analysis on 19 studies in kidney transplant recipients involving 2028 patients which showed that patients with CYP3A5*3/*3 genotype had markedly higher C/D ratios (weighted mean difference: 63.27 ng/mL per mg/kg; 95% confidence interval (CI): 50.85 - 76.30) compared with that of the combined groups of *1/*1 and

Table 4. Comparing Tacrolimus Dose, Blood Levels, Dose-adjusted Tacrolimus Concentrations and Renal Function Between Males and Females

Parameters	Gender		P
	Males	Females	
Dose, mg/kg/d			
At Hospital Discharge	0.07 ± 0.02 0.07 (0.03 - 0.14)	0.09 ± 0.03 0.09 (0.03 - 0.17)	< .05
Month 1	0.07 ± 0.02 0.07 (0.04 - 0.24)	0.09 ± 0.02 0.09 (0.05 - 0.15)	> .05
Month 3	0.06 ± 0.02 0.06 (0.02 - 0.14)	0.10 ± 0.03 0.09 (0.03 - 0.18)	< .05
Month 6	0.05 ± 0.01 0.05 (0.02 - 0.11)	0.08 ± 0.03 0.09 (0.03 - 0.19)	< .05
C, ng/mL			
At Hospital Discharge	9.79 ± 3.25 9.20 (4.70 - 23.00)	9.06 ± 3.70 8.80 (2.40 - 16.80)	> .05
Month 1	10.57 ± 3.59 10.24 (3.9 - 27.56)	8.87 ± 2.62 8.52 (2.4 - 14.77)	> .05
Month 3	9.77 ± 4.39 8.80 (5.00 - 29.35)	8.96 ± 4.19 8.10 (3.60 - 23.00)	> .05
Month 6	8.18 ± 2.04 8.00 (3.95 - 13.70)	8.22 ± 2.49 8.05 (4.50 - 14.77)	> .05
C/D, ng/mL per mg/kg/d			
At Hospital Discharge	139.41 ± 63.07 118.80 (96.49 - 168.96)	101.76 ± 54.45 88.71 (55.56 - 119.00)	< .05
Month 1	153.62 ± 64.86 148.48 (99.35 - 198.33)	97.08 ± 40.04 96.21 (62.90 - 118.29)	< .001
Month 3	164.26 ± 92.03 143.64 (96.37 - 185.48)	100.22 ± 52.52 91.20 (63.07 - 128.51)	< .001
Month 6	165.80 ± 83.98 151.31 (113.25 - 205.49)	113.72 ± 92.92 90.21 (70.93 - 131.75)	< .05
eGFR, mL/min/ 1.73m ²			
At Hospital Discharge	44.87 ± 15.34 43.50 (35.71 - 54.55)	40.14 ± 15.08 39.07 (31.00 - 47.94)	> .05
Month 1	48.58 ± 15.89 46.67 (39.03 - 58.33)	41.71 ± 16.43 39.94 (31.79 - 39.94)	< .05
Month 3	54.65 ± 19.47 55.97 (44.56 - 67.84)	51.05 ± 20.21 57.22 (35.34 - 62.20)	> .05
Month 6	57.98 ± 20.77 59.64 (46.27 - 73.85)	53.11 ± 23.11 57.95 (43.89 - 64.98)	> .05

Values are presented as mean ± SD and [median (minimum - maximum)].

C Tacrolimus trough blood concentration; D: Tacrolimus daily dose; SD: Standard deviation

Table 5. Univariate Analysis of Association Between Tacrolimus C/D Ratio During the Study Period and Demographic and CYP3A5 Genetic Characteristics of Transplant Recipients

Parameters	F	P
Gender	8.101	< .05
Age	10.336	< .05
CYP3A5 (*3/*3 vs. *1/*1 + *1/*3)	17.169	< .001
Gender * CYP3A5 Genotype	0.36	> .05

*1/*3 genotype patients. This effect was kept by time after transplant (≤ 1 month, 3 to 6 months, and 12 to 24 months after transplantation). In this meta-analysis, comparing patients with *1/*1 versus *1/*3 genotypes showed smaller difference

in C/D ratio (19.83 ng/mL per mg/kg; 95% CI: 13.86 - 25.80).²⁴

Another meta-analysis by Rojas *et al.*²⁵ revealed that C/D ratio was significantly lower in CYP3A5 expressers during the first week till month 6 after transplantation. Although CYP3A5 is assumed to be the most important genetic variant on tacrolimus metabolism,²⁶ some other variants such as CYP3A4*22 and CYP3A4*1B may also play role.²⁷

Our results showed that CYP3A5 expressers have higher risk of developing acute graft rejection episodes compared to patients with CYP3A5*3/*3 genotype. The possible explanation for this observation is that carriers of CYP3A5*1

allele have higher levels of CYP3A5 expression, higher metabolic clearance of tacrolimus, and lower tacrolimus exposure that may result in higher rate of acute allograft rejection. In addition, time to first acute rejection in CYP3A5 expressers occurred earlier compared to CYP3A5 non-expressers. However, we found no association between CYP3A5 genotypes and level of renal function (eGFR), incidence of biopsy-proven acute rejection, and delayed graft function; or 6-month allograft survival.

These results have to be taken into consideration in light of conflicting results of the literature. Some previous studies assessed association between CYP3A5 genotype and clinical outcomes of kidney transplant such as acute rejection or graft survival. MacPhee *et al.*²⁸ found that although there is no difference in rejection rates between CYP3A5 expressers and non-expressers, CYP3A5 expressers showed earlier rejection compared to non-expressers (median 7 versus 13 days, $P < .05$). This finding was in agreement with our results. The main difference between MacPhee's study and ours is lack of antibody induction in MacPhee's study compared to high-potency induction therapy with thymoglobulin in our study and dual maintenance immunosuppressive therapy in MacPhee's study compared to triple therapy in our study. These differences make the immunosuppressive regimen in their patients less potent than that in our patients.

Some other studies also reported no relationship between CYP3A5 genotype and renal function, acute allograft rejection rates, biopsy-proven acute rejection rates, and delayed graft function.^{13,14,29-31} A meta-analysis on 10 studies containing 1246 kidney transplant recipients described no difference in acute rejection rates between CYP3A5 expressers and non-expressers (OR = 0.763, 95% CI: 0.53 - 1.09).²⁴ In contrast, a larger meta-analysis on 21 studies including 2185 kidney transplant recipients revealed higher likelihood of acute allograft rejection in CYP3A5 expressers compared to non-expressers. Most of these included studies (15 out of 21) considered biopsy-proven acute rejection, while others used clinical criteria with or without biopsy criteria for definition of acute allograft rejection. Considering only patients with biopsy-proven acute rejection in meta-analysis, there was no difference in acute rejection occurrence between CYP3A5 expressers and non-expressers (OR = 1.25, 95% CI:

1.10 - 1.62), however, considering clinical suspicious for acute rejection, there was a significant higher risk of acute rejection in CYP3A5 expressers (OR = 5.04, 95% CI: 1.55 - 16.33).²⁵ Our results showed similar findings, with higher rate of suspicious acute allograft rejection in CYP3A5 expressers compared to non-expressers and no difference in biopsy-proven acute rejection between the two groups during the 6 months post transplantation.

Taken together, our results is in agreement with Chen *et al.* assumption¹¹ that tacrolimus dosing based on patients' genotype improves achieving desired blood concentrations rapidly and within initial days after transplantation compared with empiric dosing without any significant association or influence on clinically important outcomes of kidney transplantation such as acute rejection or graft survival.¹¹ By doing regular therapeutic drug monitoring during initial days after kidney transplantation and correcting sub- or supra-therapeutic range of tacrolimus blood levels by adjusting tacrolimus dose, longer-term clinical graft outcome would not be affected. Tacrolimus is usually used in combination with other immunosuppressive drugs with or without induction therapy. It seems that negative influence of sub-therapeutic tacrolimus blood concentrations during first days after transplantation may be alleviated by high potency induction therapy or high doses of corticosteroid at this time. Therefore, the cost of routine genotyping of kidney transplant recipients for CYP3A5 is not justified.

Besides genetic factors of the patients, some demographic characteristics of the recipients such as age, gender and body mass index may affect tacrolimus metabolism.³¹⁻³³ The present study showed significant correlation between C/D ratios with recipients CYP3A5 genotype, gender and age. In a study by Stratta *et al.*³¹ male sex and age of more than 60 years old were associated factors for being slow tacrolimus metabolizer. This result was in concordance with our study's finding that compared with females, male kidney transplant recipients needed significantly less daily dose of tacrolimus to reach therapeutic blood level and had higher C/D ratio during the study period. In addition, in the present study, age and C/D ratio showed significant positive correlation. Thus, the older patients had higher dose corrected blood tacrolimus concentrations indicating slower

tacrolimus metabolism with increasing age. Another study also revealed that children younger than 12 years need higher weight-based tacrolimus dose among both CYP3A5 expressers and non-expressers.³⁴

It has been shown that higher doses of methylprednisolone cause decrease in C/D ratio of tacrolimus independent of CYP3A5 polymorphism.^{31,34} In the present study all patients (CYP3A5 expressers and non-expressers) received similar corticosteroid pulse and taper down regimen, therefore; it is expected that the influence of steroid dose on tacrolimus metabolism exert less confounding effect on our results.

This study has some limitations including small sample size, short period of patients' follow-up, and no assessment of the area under the concentration-time curve of tacrolimus as an index of tacrolimus exposure for comparing between CYP3A5 expressers and non-expressers.

Although patients who were taking medications known to interact with CYP3A5 (except for methylprednisolone and prednisolone) were excluded from this study, however; we did not take into account other factors that could affect tacrolimus pharmacokinetics such as haematocrit, liver function, and serum albumin level.

In conclusion, the results of our study revealed that CYP3A5 genetic polymorphism is significantly associated with tacrolimus pharmacokinetics. Patients who express at least one allele of CYP3A5*1, known as CYP3A5 expressers; needed significantly higher daily dose of tacrolimus to reach desired therapeutic blood concentration and had less dose-corrected blood concentrations than CYP3A5 non-expressers. CYP3A5 expressers also showed higher rate of clinically suggested acute rejection and earlier time to first acute rejection episode after kidney transplantation compared with patients homozygous for CYP3A5*3. However, Level of kidney function, grafts' survival, and the rates of biopsy proven acute allograft rejection, and delayed graft function did not differ between CYP3A5 expressers and non-expressers. Therefore, it is not the time to routinely assess kidney transplant recipients for CYP3A5 genetic polymorphism before transplantation.

ACKNOWLEDGEMENT

Authors appreciate the valuable helps of nursing

staff of kidney transplant ward of Imam-Khomeini Hospital Complex (Mrs. Shahin Derafshi, Mrs. Fatemeh Haddadi and Mrs. Monireh Derakhshan) and Milad Hospital (especially Mrs. Neda Arshideh). They also wish to thank Dr. Maryam Ghadimi and Dr. Mojdeh Gohari for their cooperation in transplant recipients' follow-ups.

FUNDING

This research is part of the Clinical Pharmacy thesis that has been supported by Tehran University of Medical Sciences (grant number: 94-04-33-30643).

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Received April 2019

Revised July 2019

Accepted September 2019