Original Article

Influence of CYP3A5 and ABCB1 Polymorphism on Tacrolimus Drug Dosing in South Indian Renal Allograft Recipients

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Abstract

Introduction: Tacrolimus blood levels are influenced by polymorphisms involving Cytochrome 3A subfamily (CYP3A5) and P-Glycoprotein (ABCB-1) genes. However, their role in transplant outcomes was less studied in South Indian population. We studied the prevalence and impact of these polymorphisms in renal transplant recipients from South India. **Methods:** An analysis of CYP3A5, ABCB1 genotype done in 101 renal transplant recipients by polymerase chain reaction was correlated with blood tacrolimus trough levels (CLIA method), weight, concentration/dose (L/D) ratio, incidence of biopsy proven early acute rejections, and tacrolimus toxicity. **Results:** Prevalence of CYP3A5*1/*1, *1/*3 and *3/*3 and ABCB1 (3435C>T) TT, CT, CC genotypes were 12 (11.9%), 48 (47.5%), 41 (40.6%) and 16 (15.8%), 45 (44.6%), 40 (39.6%), respectively. Mean tacrolimus level, median concentration/dose (L/D) ratio were significantly lower in homozygous (CYP3A5*1/*1-6.01 ng/mL; 48.99 ng/mL/mg/kg/day) and heterozygous expresser group (CYP3A5*1/*3-5.84 ng/mL; 68.93 ng/mL/mg/kg/day) when compared with nonexpresser group [CYP3A5*3/*3-7.46 ng/mL *(P* < 0.001);181.3 ng/mL/mg/kg/day (*P* < 0.05]. No significant differences observed between the ABCB1 genotypic groups. Incidence of early acute rejections (30% vs. 9.76%; *P* 0.016) and tacrolimus-related toxicity (14.6% vs. 5%; *P* 0.039) were significantly higher in CYP3A5 expressers and nonexpressers, respectively. No correlation observed between the ABCB1 polymorphisms between rejection episodes or tacrolimus renal toxicity. Among 101 patients, 40.6% were non-expressers (poor metabolizers) (*3/*3). **Conclusions:** CYP3A5 polymorphisms correlated with tacrolimus dose requirements and blood levels, incidence of early acute rejection, and tacrolimus nephrotoxicity. CYP3A5 polymorphism analysis prior to renal transplant will aid more precise early tacrolimus dose calculation to balance between rejection and toxicity.

Keywords: *Polymorphisms, rejection, renal transplant, tacrolimus, tacrolimus toxicity*

Introduction

Tacrolimus is the vital component of renal transplant immunosuppressive therapy. However, because of its narrow therapeutic range, trough level monitoring is important for the prevention of its toxicity as well as rejection. Tacrolimus is metabolized by the liver cytochrome P450 CYP3A5 enzyme family.[1] CYP3A5 is expressed in the liver and catalyses the metabolism of tacrolimus.[2] CYP3A5 is also expressed in the small intestine and is thought to participate in the oral clearance of tacrolimus. A polymorphism in intron 3 of the *CYP3A5* gene was found to influence the expression of this enzyme; *CYP3A5*3* allele (G at position 6986) produces a cryptic splice site and encodes an abnormal spliced mRNA with a premature stop codon, while *CYP3A5*1* allele (A at position 6989) produces a normal mRNA, resulting in a high expression of this enzyme in the intestine and in the liver.[3,4] Previous studies showed that renal graft recipients homozygous for the wild-type allele *CYP3A5*1* required higher tacrolimus doses to achieve the target trough concentrations compared with carriers of the variant allele *3.[5-8] Individuals possessing at least one *CYP3A5*1* allele (CYP3A5 expressers) achieved twofold lower tacrolimus concentrations/dose ratio compared with *CYP3A5*3/*3* homozygous (CYP3A5 nonexpressers).[9] Polymorphisms in intron 3 of this gene affects the metabolic activity of this enzyme.

The oral bio availability is also determined by the P-glycoprotein, an efflux pump encoded *by ATP Binding Cassette 1 (ABCB1)* gene, expressed in the apical membranes of epithelial cells, such as liver, kidney, and intestine, and it contributes to the elimination of drugs into the bile and urine. P-glycoprotein acts in synergy with

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the CYP3A subfamily in limiting intestinal absorption of various drugs.[10] Genetic polymorphisms in exon26 (3435C>T) were correlated with cellular expression level of P-glycoprotein in relation with ABCB1 mRNA stability and/or the protein's timing of cotranslational folding.[11] The effect of this *ABCB1* polymorphisms on tacrolimus oral bioavailability remains controversial.[8] The effect of *CYP3A5* and *ABCB1* single nucleotide polymorphisms (SNPs) on tacrolimus pharmacokinetics are not well studied in South Indian population.

The purpose of this study was to identify the prevalence of CYP3A5, ABCB1 polymorphisms in South Indian kidney transplant recipients and to determine the effect of *CYP3A5*, *ABCB1*gene polymorphisms on serum tacrolimus concentration (L/D ratio), incidence of acute rejections and Calcineurin inhibitortoxicity.

Patients and Methods

In total, 101 renal allograft recipients consented to participate in this study. The study population was recruited from the renal transplant outpatient clinic of our institution, between April 2017 and September 2017. Only patients with tacrolimus-based immunosuppressive treatment with stable graft function of >6-month duration were eligible to participate in the study. Patients with ongoing rejections, infections, with drugs that interfere with the tacrolimus levels, and abnormal liver function tests were excluded from the study. Our immunosuppressive protocol consisted of initial daily dose of tacrolimus was 0.1 mg/kg, given twice daily. Then, the dose was adjusted to obtain a trough blood concentration between 8 and 10 ng/mL for the first 6 months, followed by between 5 and 8 ng/ml. All patient's serum tacrolimus were obtained from their records, which were taken at the sixth month of post-transplant period. All whole blood tacrolimus trough concentrations were measured by chemiluminescence immunoassay (CLIA) from a single laboratory. Tacrolimus daily dose and patient's body weight were obtained from records of all patients at the time of trough level estimation. Level/dose ratio (ng/mL/mg/kg/day) was calculated for all patients. Peripheral blood samples for genotypic analysis obtained from all patients, during their regular visit to nephrology OPD (2 mL of Ethylenediaminetetraacetic acid preservative added and transported at 4°C temperature for genotyping). Biopsy-proven early acute rejection episodes (BPAR) reported during the first month of post-transplantation and the incidence of biopsy-proven CNI toxicity (biopsy findings correlated with clinical aswell as the blood C0 levels) were obtained retrospectively from patient's medical files. BPAR was considered for at least Banff 1 in severity according to classification of Banff '05.[12] Study was approved by the ethics committee of our institution. Protocols followed according to the ethical guidelines of the 1975 Helsinki Declaration. All patients were explained in detail about the study in their vernacular language and written consent obtained from all patients. The patients who participated in this study did not incur any expenditure.

Genomic DNA extraction and allele-specific polymerase chain reaction with melting point analysis

Genomic DNA was extracted from 0.2mL of peripheral blood by silica column-based DNA extraction kit (Cat# 51104, QiaAmp Blood DNA kit, Qiagen, Germany) as per manufacturer's protocol. Genotyping for CYP3A5 (A6986G)(rs776746) and ABCB1 (C3435T) (rs1045642) polymorphisms were performed by allele-specific polymerase chain reaction (PCR) in combination with melting point analysis (ASPCR-MPA). Briefly, 50ng of genomic DNA was subjected to allele-specific PCR with following primer pairs (Eurofins, Bangalore):

CYP3A5

- Forward primer: CACTTGATGATTTACCTGCCTTC
- Reverse primer-wild-type allele specific: GGTCCAA ACAGGGAAGAGATAT
- Reverse primer- mutant allele specific: GGTCCAA ACAGGGAAGAGATAC.

MDR1 (ABCB 1)

- Forward primer: ACTATAGGCCAGAGAGGCTGC
- Reverse primer wild-type allele specific: GTGGTGTCACAGGAAGAGCTT
- Reverse primer mutant allele specific: GTGGTGTCACAGGAAGAGCTC.

As described earlier by Ashavaid *et al*.,[13] the CYP3A5 primers produce a 218-bp Amplicon, whereas MDR1 primers produced 134-bp Amplicon. The allele-specific real-time PCR amplification of samples were performed with Type-IT HRM kit (cat #206542, Qiagen) in a Qiagen Rotor Gene Q real time PCR system (Qiagen) under following conditions: after an initial denaturation at 94°C for 4 min, samples were subjected to 35 cycles of denaturation at 94°C for 30 s, primer annealing at 60°C for 30 s, and primer extension at 72°C for 30 s. Following the amplification cycles, the samples were subjected to melting point analysis with 0.1°C/s to confirm the specificity of amplification and distinguish the amplification curve from possible primer dimers or nonspecific amplifications.

Statistical analysis

Data are described as mean ± SD and median ± 2SD for all quantitative estimates. To estimate the effect of *CYP3A5* and *ABCB1* genotypes on tacrolimus daily dose, we used one-way analysis of variance (ANOVA) test. For concentration/dose ratio of tacrolimus, we used Kruskal-Wallis test. Tacrolimus trough blood levels, L/D ratio and rejection episodes between two groups (expresser vs.nonexpresser) were compared by means of independent *t*-test. Chi-square test was used to test the risk of acute rejection and CNI toxicity between the three genotype groups. Fisher's exact test was used to test the risk

of CNI nephrotoxicity in different groups. Statistical analysis was done using R- software version 3.4.2 (Stanford). For all the tests, *P* value < 0.05 was considered to be statistically significant.

Results

In total, 101 renal graft recipients with tacrolimus-based immunosuppressive treatment were included in the study. There were 85 (84.2%) men and 16 (15.8%) women. The mean age was 32 (27–38.5) years and body weight was 60.4 ± 13 kg. All patients were of South Indian origin. Most common Etiology for ESRD was unknown (73.3%); glomerular diseases accounted for 21% of ESRD (IgAN-7.9%, FSGS-5%). 70.3% were living related transplants and deceased donor graft recipients accounted for 29.7%. The median post-transplant duration was 32 months (16.5-53). Mean serum creatinine was 1.36 ± 0.3 mg/dL. About 34.7% patients received induction therapy (ATG- 27, Basiliximab- 8). There was no significant differences noted in terms of age, sex, donor type (deceased or live), and induction agent usage between the CYP3 groups. However, we were not able to analyze the HLA match in the deceased donor and spousal donor transplants. All patients were on tacrolimus-based immunosuppression in combination with steroids and mycophenolate mofetil (*n* = 99), or steroids and azathioprine (*n* = 02). Twenty-two (21.8%) patients had early BPARs within the first month of transplantation. Overall, 8.9% (9 patients) had biopsy evidence of tacrolimus-related renal toxicity (within first year of transplantation). 5 patients in living donor group and 4 patients in deceased donor group had delayed graft function (7.1% and 13.34%). Mean donor age in living and deceased donor group was 51.6 years and 43 years (p < 0.01) respectively. 18.8% patients had infection episodes and required admission in the first post-transplant year and NODAT was present in 19 (18.8%) patients.

The CYP3A5*1/*1,*1/*3, and *3/*3 genotypes were detected in 12 (11.9%), 48 (47.5%), and 41 (40.6%) of the 101 graft recipients, respectively. The ABCB1 (3435C>T) TT, CT, and CC genotypes were in 16 (15.8%), 45 (44.6%), and 40 (39.6%), respectively.

Effect of CYP3A5 genetic polymorphism on tacrolimus daily dose and concentration/dose ratio

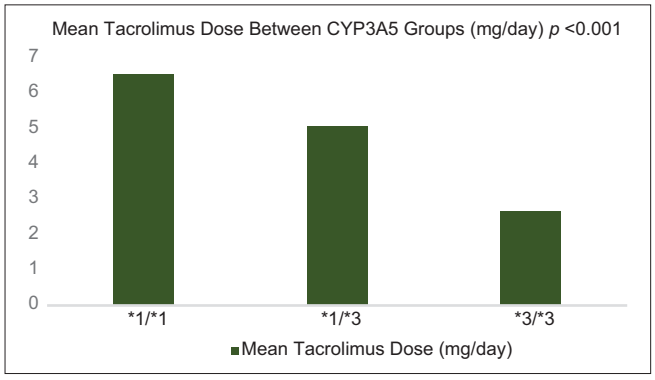
The daily tacrolimus dose requirement was much higher for CYP3A5 expresser (*1/*1 and *1/*3) groups when compared with the CYP3A5 non-expresser (*3/*3) group [Graph 1]. Mean tacrolimus level in the CYP3A5*1/*1, CYP3A5*1/*3, and CYP3A5*3/*3groups were 6.01 ng/mL (SD = 4.53 ng/mL), 5.84 ng/mL (SD = 1.78 ng/mL), and 7.46 ng/mL (SD = 2.99 ng/mL), respectively, with the *P* value of <0.05 [Graph 2]. The heterogeneous expresser group (*1/*3) had lower mean Tac level when compared with the homogenous expresser group (*1/*1), which may be because of lesser patients in homogenous group (12 vs. 48) and the difference is not statistically significant (*P* > 0.05).Tacrolimus level difference between expressers and nonexpressers were significant when compared by using one-way ANOVA test. CYP3A5*1/*1,*1/*3 groups had significantly lower median concentration/dose (L/D) ratio than the CYP3A5*3/*3 non-expresser group (48.99, 68.93, and 181.3 (ng/ml/mg/kg) with the *P* value of <0.001) when compared by Kruskal-Wallis test [Graph 3]. Incidence of early acute rejections and CNI toxicity were compared between expresser (CYP3A5 *1/*1, *1/*3) and non-expresser genotypic groups (CYP3A5*3/*3). Expressers had significantly higher rejection rates when compared with nonexpressers (30% vs. 9.76%; *P* < 0.05). Meanwhile, nonexpressers had higher incidence of biopsy-proven CNI toxicity when compared with expressers (5% vs. 14.6%; *P* < 0.05) [Table 1].

Effect of ABCB1 (MDR1) genetic polymorphisms on tacrolimus daily dose and concentration/dose ratio

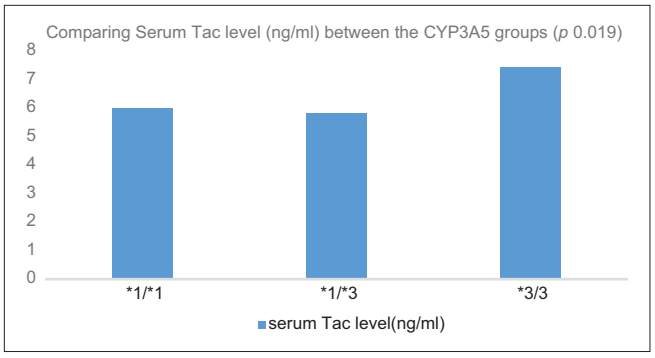
Our analysis showed that *ABCB1* polymorphisms did not have significant effect on tacrolimus daily dose and concentration/dose ratio. Incidence of early acute rejections and CNI toxicity were not significantly different between ABCB1 (3435C>T) - C/C, C/T, T/T genotypes [Table 2].

Discussion

Tacrolimus is a potent immunosuppressive drug used in solid organ transplantation. But, it has got a narrow therapeutic

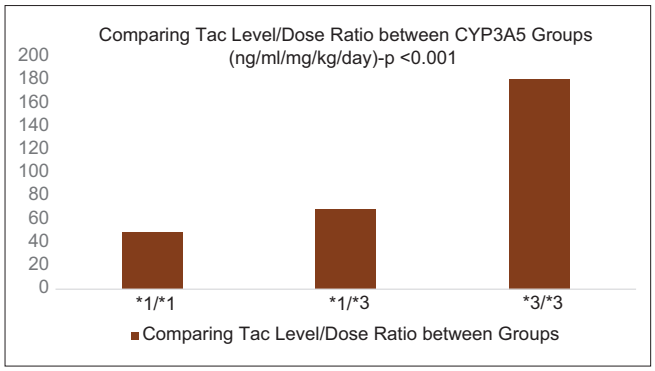


Graph 1: Comparing the tacrolimus dose requirements between the CYP3A5 groups



Graph 2: Comparing the blood tacrolimus levels between the CYP3A5 groups

range, which is further complicated by wide variation in intra-individual and inter-individual bioavailability of drug. Tacrolimus is metabolized by CYP3A5 enzymes in liver and small intestine. Genetic polymorphisms in CYP3A5 are found to influence the inter-individual variability in tacrolimus trough blood levels.[2] The prevalence of CYP3A5*3*3 (CYP3A5 non-expressers) in our study was 40.6%. This is in contrast to study by Lina Quteineh *et al*. from France, which showed the prevalence of non-expresser genotype (CYP3A5*3*3) was 75%.[14] Another study by Ashavaid *et al*. from North India showed the prevalence of 40%.[13] Meaning that Indian patients have more wild-type allelic variants of CYP3A5.



Graph 3: Comparing the L/D ratio between the CYP3A5 Groups

In our study, we evaluated the effect of CYP3A5 genetic polymorphisms on tacrolimus daily dose requirements in a cohort of kidney transplant recipients. Our results show that carriers of at least one active allele (CYP3A5*1) needed significantly higher doses of tacrolimus compared with patients homozygous for CYP3A5*3 (CYP3A5 non-expressers). This result relies on the fact that carriers of CYP3A5*1 allele exhibit high levels of CYP3A5 expression and enzymatic activity, leading to higher daily dose requirement to achieve normal trough levels of tacrolimus. Such results have been previously replicated in the literatures concerned to this polymorphism.[5-9] At 6 -month post-transplantation, patients homozygous for *CYP3A5*1* needed more than threefold higher doses of tacrolimus compared with patients homozygous for the variant allele *CYP3A5*3*, and patients heterozygous

Table 1: Comparison of CYP3A5 polymorphisms with the characteristics

Characteristics	CYP3A5*1/*1	CYP3A5*1/*3	CYP3A5*3/*3	*P*
Number of patients	12	48	41	0.12
Recipient age in years (mean)	34.16	32.2	32.58	0.081
Donor age in years (mean)	47.91	49.92	49.13	0.11
Male:Female	10:2	38:10	37:4	0.11
Live donors	58.33%	70.83%	73.17%	0.071
Mean creatinine (mg/dL)	1.27	1.31	1.45	0.088
Tacrolimus dose (mg/day) (mean±SD)	**6.54(±2.17)**	**5.08(±1.85)**	**2.66(±1.21)**	**<0.001**
Tacrolimus level (ng/mL)-(mean)	**6.01(±4.54)**	**5.84(±1.78)**	**7.46(±2.99)**	**0.019**
L/D ratio (ng/mL/mg/kg/day)	**48.99**	**68.93**	**181.3**	**<0.001**
Induction treatment	50%	35.42%	29.27	0.986
Rejections	**3 (25%)**	**15 (31.3%)**	**4 (9.76%)**	**0.047**
	18 (30%)		**4 (9.76%)**	**0.016**
CNI toxicity	1	2 (4.2%)	6 (14.6%)	0.158
	3 (5%)		**6 (14.6%)**	**0.039**

CYP3A5: Cytochrome P450 family 3 subfamily A member 5

Table 2: Comparison of ABCB1 polymorphisms with the characteristics

Characteristics	ABCB-CC	ABCB-CT	ABCB-TT	*P*
Number of patients	40	45	16	0.061
Recipient age in years (mean)	32.22	33.82	30.06	0.128
Donor age in years (mean)	48	50.1	48.13	0.28
Male: Female	33:7	40:5	4:12	0.212
Live donors	60%	80%	68.75%	0.741
Mean creatinine (mg/dL)	1.36	1.35	1.4	0.311
Tacrolimus dose (mg/day) (mean±SD)	4.5(±2.16)	4.23(±2.17)	3.83(±2.29)	0.575
Tacrolimus level (ng/mL) (mean±SD)	6.46(±3.15)	6.31(±2.15)	7.27(±3.59)	0.499
L/D ratio (ng/mL/mg/kg/day) (median: IQR)	74.45	87.75	116.71	0.26
Induction treatment	42.5%	31.11%	25%	0.10
Rejections	9 (22.5%)	9 (20%)	4 (25%)	0.65
CNI toxicity	3 (7.5%)	4 (8.95)	2 (12.5%)	0.32

ABCB1: ATP binding cassette subfamily B member1

for this polymorphism needed intermediate doses to achieve the same target trough concentration. Patients with *CYP3A5*3/*3* genotype achieved fourfold higher concentration/dose (L/D) ratio compared with patients with *CYP3A5*1/*1* genotype.

We also evaluated the risk of BPAR during the first month post-transplantation. We found that patients with CYP3A5*1/*3 and *1/*1 genotypes had significant higher incidence of acute graft rejection episodes compared with CYP3A5*3 homozygotes. This observation is also seen in the study by Sreeja Nair *et al*.[15] This observation is in agreement with the fact that carriers of the wild-type allele (*CYP3A5*1*) have higher levels of CYP3A5 expression, higher metabolic clearance of tacrolimus, low trough concentrations, and, therefore, acute rejection. We noticed a significant difference of tacrolimus trough concentration between carriers of at least one active allele (*CYP3A5*1*) and patients with *CYP3A5*3/*3* genotype. Additionally, tacrolimus trough concentration of patients with at least one active allele (*CYP3A5*1*) was at the lower limit of the targeted trough levels and could confirm the higher risk of acute rejection in this group. Few rejection episodes occurred after the first month of transplantation (three episodes within the *CYP3A5*3/*3* group), so the therapeutic tacrolimus level are more crucial during the first month after transplantation.

In our study, we were not able to get the details of HLA mismatches between donor and recipients (especially in cadaveric renal transplants), which is also an important risk factor for acute rejection. When compared to the living donor graft recipients, deceased donor graft recipients had no inceased incidence of early acute rejections (21.1% vs 23.3%) or CNI toxicity (8.5% vs 10%).

We found significant correlation between the development of tacrolimus-related nephrotoxicity and *CYP3A5* genetic polymorphism. This finding could be explained by the fact that the patients with *CYP3A5*3/*3* genotype had higher incidence of toxicity due to higher trough levels and higher concentration/dose ratio. We did not find an association between tacrolimus daily dose and tacrolimus-related nephrotoxicity.

We found no association between *ABCB1* exon26 (3435C>T) genetic polymorphisms and tacrolimus daily requirements and concentration/dose ratio. Similar findings were observed by in the study by Lina Quteineh *et al*.[14] This is in agreement with most of the published data evaluating this polymorphisms.[8,10,16] We also found no association between *ABCB1* exon26 (3435C>T) genetic polymorphisms and incidence of rejection or CNI toxicity. This is in contrast to the study from North India by Ashavaid *et al*. who observed significantly higher tacrolimus levels and concentration/dose ratio in *ABCB1* exon26 (3435C>T) homozygous mutants (T/T).

Previous study by Mohan Patel *et al*.[17] studied the influence of CYP3A5 polymorphism on tacrolimus drug dosing in North Indian renal allograft recipients. To our knowledge, this is the largest study to show the association between CYP3A5 genetic polymorphism and tacrolimus drug level in South Indian population and also the first study to evaluate the association between the ABCB1 genetic polymorphism in South Indian cohort. Our study is a cross-sectional study, which is relying on the retrospective data. Large-scale prospective studies will be helpful in consolidating these findings.

Conclusions

Our results confirm that CYP3A5 genetic polymorphism is an important factor in determining tacrolimus daily requirements. It was shown from our study that CYP3A5 genetic polymorphism is implicated in the risk of developing acute rejection episodes and tacrolimus-related renal toxicity. As per our study, ABCB1 polymorphisms have not been shown to influence the serum tacrolimus concentrations and dose requirements.

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Conflicts of interest

There are no conflicts of interest.

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