

# Clinical Efficacy and Safety of Tacrolimus in Pakistani Living Donor Liver Transplant Recipients

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## ABSTRACT

**Objective:** To evaluate the association between tacrolimus trough levels and dosage in Pakistani patients undergoing live donor liver transplantation (LDLT), and the efficacy and adverse effects at different tacrolimus trough levels and dosages.

**Study Design:** An observational study.

**Place and Duration of Study:** Shifa International Hospital, Shifa Tameer-e-Millat University, Islamabad and Basic Medical Sciences Institute, Karachi, from September 2016 to October 2018.

**Methodology:** Sixty liver transplant recipients were included. Demographics, clinical data, tacrolimus trough levels and doses were monitored as per routine protocol. Electrochemiluminescence immunoassay (ECLIA) was used to measure tacrolimus trough levels. Acute cellular rejection (ACR), sepsis and other adverse events were monitored at different tacrolimus trough levels in early post-transplantation period.

**Results:** Mean age of transplant recipients was 49.1 ±10.6 years. Mean tacrolimus trough levels were 6.1 ±2.2 ng/ml and mean dose was 0.94 ±0.3 mg. Sepsis (27%) psychosis (20%), seizures (10%), and renal insufficiency (13%) were the most common adverse effects. Acute cellular rejection (ACR) was observed in 15% patients. Patients with sepsis had significantly high mean tacrolimus levels of 7.7 ±2.5 ng/ml versus 5.5 ±1.9 ng/ml (p=0.001). Mean tacrolimus trough levels in patients with ACR were significantly lower (4.05 ±1.6 ng/ml vs. 6.43 ±2.2ng/ml, p=0.003). None of the patients with a single tacrolimus trough level >10 ng/ml experienced ACR.

**Conclusion:** A tacrolimus trough level between 5 to 7.5 ng/ml appears to be safe in Pakistani liver transplant recipients significantly minimising the risk of ACR and other adverse events.

**Key Words:** Tacrolimus, Liver transplant, Adverse effects, Immunosuppression.

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## INTRODUCTION

Liver transplantation is a life-saving treatment for patients with end-stage liver diseases.<sup>1</sup> In the United States alone, liver transplantation was performed on eight thousand patients suffering from end-stage liver disease in 2017.<sup>2</sup> Pakistan with its population over 180 million has a prevalence of 2.4% for hepatitis B virus (HBV) and 6.2% for hepatitis C virus (HCV) infection. These are major risk factors for liver cirrhosis and hepatocellular carcinoma.<sup>3,4</sup> Due to high burden of HCC and liver failure, living donor liver transplant (LDLT) is a viable treatment option for these patients.<sup>5</sup>

Alongside advances in surgical technique and post-operative supportive care, the use of potent immuno-

suppressive agents has made this procedure safer by preventing and treating acute and chronic rejection.<sup>6</sup> Calcineurin inhibitors (CNI) based drug regimens are mainstay of immunosuppressive protocols in patients undergoing liver transplantation. Both tacrolimus and cyclosporine are calcineurin inhibitors and bind to their specific ligands, immunophilin FK-binding protein and cyclophilin respectively; which eventually suppress immune response by inhibiting activation of T-cell specific transcription factor NF-AT and transcription of multiple cytokine genes.<sup>7,8</sup>

Calcineurin inhibitors have a narrow therapeutic index, high inter- and intra-individual pharmacokinetic variability, irregular oral bioavailability and require therapeutic drug monitoring (TDM) in order to optimise clinical outcomes after transplantation.<sup>9,10</sup> Individual genetic variations and single nucleotide polymorphism in CYP3A4, CYP3A5, PPARA and ABCB1 genes which encode for cytochrome P450 enzymes and p-glycoprotein efflux pumps may significantly compromise the bioavailability of calcineurin

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inhibitors thereby altering blood levels and increasing the chances of acute and/or chronic rejection.<sup>11</sup>

Tacrolimus is generally preferred over cyclosporine for immunosuppression in liver transplantation due to multiple reasons, which include higher potency of tacrolimus and less rejection episodes.<sup>8,12</sup> However, early employment of tacrolimus in the post-transplantation periods requires vigilant monitoring of drug concentration as serious adverse effects as neurotoxicity and nephrotoxicity can develop during optimization of tacrolimus therapy, especially at higher plasma levels.<sup>13</sup>

The aim of the current study was to evaluate the relationship between tacrolimus blood concentrations and dosage in Pakistani patients undergoing LDLT, and explore the efficacy and tolerability of tacrolimus in terms of immunosuppression and adverse effects using tacrolimus trough levels.

## METHODOLOGY

The study was conducted at Shifa International Hospital, Islamabad, from September 2016 to October 2018. The protocol of the study was approved by the Institutional Review Board. It was an observational prospective study and 60 liver transplant recipients were enrolled in the study after obtaining written informed consent. All LDLTs were performed after approval from human organ transplant authority (HOTA) and ethical committee of the hospital. Baseline characteristics including demographics, medical history, and laboratory profile were recorded for all transplant recipients. Details of the evaluation process of the donors and recipients have been reported elsewhere.<sup>14,15</sup>

Immunosuppression was initiated approximately 12 hours after the transplantation. As a routine, oral or nasogastric route was used. The standard immunosuppressive agents used at our centre were tacrolimus and steroids; and the initial dose of tacrolimus on the first day after liver transplant was 0.5 mg/day. Venous blood samples of patients were collected in 5 mL EDTA plastic tubes to measure tacrolimus trough concentrations. The trough levels of tacrolimus were measured as routine protocol on day 3, 6, 9 and 12 using electrochemiluminescence immunoassay (ECLIA). Adjustments in the dose of tacrolimus were made on the basis of the standard of care blood level monitoring of tacrolimus, other clinical indices such as AST, ALT or reporting of adverse effects. Subjects were evaluated until 14 days post-transplantation. Daily laboratory measurements of AST, ALT, alkaline phosphatase, GGT, total bilirubin, serum creatinine, BUN, serum potassium, serum magnesium, blood glucose, albumin, and hematocrit were performed.

In first two weeks post-transplant, tacrolimus trough levels of 7-8 ng/ml were considered acceptable. In patients with renal insufficiency, other immunosuppressant such as

mycophenolate mofetil (MMF) were considered. Outcome was assessed, based on mean tacrolimus trough levels and its impact on adverse outcomes and ACR. Patients in whom tacrolimus trough levels were more than 10 ng/ml at least once were also assessed separately.

Collected data was analysed by appropriate statistical methods using Statistical Package for Social Sciences (SPSS V. 22) programme. Students' 't' test was applied for comparison between different groups, continuous variables were specified in mean and SD; whereas categorical variables were described by frequencies, percentages, and computed by Chi-square and Fisher's exact test. A p-value less than 0.05 was considered statistically significant.

## RESULTS

Sixty liver transplant recipients were included, out of which 45 (75%) were males and 15 (25%) were females. Mean age of liver transplant recipients was 49.1 ±10.6 years and male to female ratio was 3:1. The most common etiology for end-stage liver disease was HCV in 25 (41.7%) and HBV in 10 (16.6%) patients. Mean tacrolimus dose administered was 0.94 ±0.3 mg/day and mean tacrolimus trough levels were 6.1 ±2.2 ng/ml. The demographic and clinical data is summarised in Table I.

**Table I:** Demographic and clinical data.

Variables	Number Percentage	(%)
Gender		
Male	45	75
Female	15	25
Etiology		
HCV	25	41.7
HBV, ESLD	10	16.7
HDV	8	13.3
HBV, HDV	8	13.3
Cryptogenic liver cirrhosis, HCC	2	3.3
Other*	7	11.7
Total	60	
Ethnicity		
Punjabi	21	35
Pathan	17	28.3
Sindhi	09	15
Urdu speaking	07	11.7
Balochi	4	6.7
Gilgit-Baltistan	2	3.3
	Mean	±SD
Age (years)	49.1	±10.6
Height (cm)	168.22	±7.7
Weight (kg)	74.1	±14.5
BMI (kg/m <sup>2</sup> )	26.7	±5.2
Mean tacrolimus dose (mg/day)	0.94	±0.292
Mean tacrolimus trough levels (ng/ml)	6.1	±2.2
Mean tacrolimus trough levels after dose 1 (ng/ml)	4.0	±3.4
Mean tacrolimus trough levels after dose 2 (ng/ml)	6.0	±3.7
Mean tacrolimus trough levels after dose 3 (ng/ml)	6.8	±3.3
Mean tacrolimus trough levels after dose 4 (ng/ml)	7.5	±2.8

\*Other (Acute-on-Chronic Liver Failure=1, Acute liver failure =1, Budd-Chiari Syndrome 1, HCV, HBV, HDV, HCC=1, NBNC liver cirrhosis=1, Primary Sclerosing Cholangitis=1, HBV, CLD=1).

**Table II:** Mean tacrolimus trough levels in patients with adverse effects and acute cellular rejection (n=60).

Variables	No. of patients (%)	Tacrolimus trough levels (ng/ml) (Mean $\pm$ SD)	p-value
Male recipients	45 (75)	6.10 $\pm$ 2.3	0.87
Female recipients	15 (25)	6.00 $\pm$ 1.9	
Sepsis (+)	16 (27)	7.7 $\pm$ 2.5	0.001*
Sepsis (-)	44 (73)	5.5 $\pm$ 1.9	
Psychosis (+)	12 (20)	7.2 $\pm$ 2.7	0.04*
Psychosis (-)	48 (80)	5.8 $\pm$ 2.1	
Seizures (+)	6 (10)	7.03 $\pm$ 3.0	
Seizures (-)	54 (90)	5.97 $\pm$ 2.2	0.28
Renal insufficiency (+)	8 (13.3)	7.97 $\pm$ 2.9	0.01*
Renal insufficiency (-)	52 (87)	5.78 $\pm$ 2.0	
Acute cellular rejection (+)	9 (15)	4.05 $\pm$ 1.6	0.003*
Acute cellular rejection (-)	51 (85)	6.43 $\pm$ 2.2	

**Table III:** Comparison of adverse effects, sepsis and acute cellular rejection in patients with tacrolimus trough levels greater than 10 ng/ml (n=60).

	Tacrolimus levels >10 ng/ml (n=24)	Tacrolimus levels $\leq$ 10 ng/ml (n=36)	p-value
Adverse effects (+)	13 (54.1)	4 (11.1)	<0.001*
Adverse effects (-)	11 (45.9)	32 (88.9)	
Sepsis (+)	12 (50)	4 (11.1)	0.002*
Sepsis (-)	12 (50)	32 (88.9)	
Acute cellular rejection (+)	0	9 (25)	0.008*
Acute cellular rejection (-)	24 (100)	27 (75)	

There was a significant difference in mean tacrolimus levels in patients with and without ACR [4.05  $\pm$ 1.6 ng/ml versus 6.4  $\pm$ 2.2 ng/ml, (p=0.003)] as shown in Table II. Similarly, a significant difference in mean tacrolimus levels was observed in patients with and without sepsis [7.7  $\pm$ 2.5 ng/ml versus 5.5  $\pm$ 1.9 ng/ml (p=0.001)], psychosis [7.2  $\pm$ 2.7 ng/ml versus 5.8  $\pm$ 2.1 ng/ml (p=0.04)] and renal insufficiency [7.9  $\pm$ 2.9 ng/ml versus 5.7  $\pm$ 2 ng/ml (p=0.01)]. Pearson correlation test found no impact of BMI on tacrolimus trough levels.

Twenty-four out of 60 patients had tacrolimus trough levels more than 10 ng/ml at least once during 14 days post transplant. Episodes of adverse events 13/24 (54.1%) versus 4/36 (11.11%, p<0.001), sepsis 12/24 (50%) versus 4/36 (11.11% p=0.002) and ACR 0 versus 9/36 (25%, p=0.008) were significantly more common in these patients are shown in Table III.

## DISCUSSION

A high number of male transplant recipients in the current study is in agreement with other studies which have highlighted different factors for the uneven gender distribution for transplant candidacy. These include prevalence of HCC in males and imperfect model for end-stage-liver disease (MELD) score which uses serum creatinine. Levels of serum creatinine are higher in males because of higher body muscle mass.<sup>16-18</sup> Nevertheless, there was no significant effect of gender and BMI on mean tacrolimus trough levels.

The major ethnic groups in our study population were Punjabi and Pushto speaking. This by no means suggests that liver failure or HCC is more prevalent in this patient group. In fact, we believe that easy access to the transplant centre due to its location in northern part of the country was responsible for this ethnic distribution.

Titrating the dose of tacrolimus to optimum levels in liver transplant recipients is a challenging task as lower than therapeutic plasma levels can result in ACR; whereas, higher plasma levels can lead to serious adverse effects such as nephrotoxicity, neurotoxicity and increased risk of acquired infections. In this study, tacrolimus was titrated to achieve optimal levels in plasma and consequently the dosages of tacrolimus varied in different patients. The mean dosage of tacrolimus administered for the first 14 days after liver transplant in all patients was 0.94 mg/day. Mean tacrolimus trough levels reported in this study are 6.1  $\pm$ 2.2 ng/ml. These levels are considerably lower than the mean values reported from other studies conducted in China (11.3  $\pm$ 4.8 ng/ml) and India (7.3  $\pm$ 2.9 ng/ml) in live donor liver transplant patients.<sup>18,19</sup> Variable metabolism of tacrolimus due to underlying genetic polymorphism could be possible reason for different tacrolimus trough levels in different populations.

Sepsis, psychosis, seizures and renal insufficiency appeared to be the common adverse effects and mean trough tacrolimus levels in these recipients were lower than other studies reporting similar adverse effects. In different studies, occurrence of adverse effects was reported at high tacrolimus trough concentration or even higher than the therapeutic concentration of 15 ng/ml.<sup>19,20</sup> In this study, 27% recipients developed sepsis and mean tacrolimus trough levels in these patients were significantly higher than other recipients without acquired infection. Similarly, recipients who developed renal insufficiency and neurotoxicity also had significantly higher mean tacrolimus trough levels. These results are consistent with results of other transplant programmes which have reported similar frequency of sepsis and other tacrolimus related adverse events.<sup>19,21-23</sup> However, the unique point in the current study is the lower cut off level at which adverse events were noted.

This study reported 15% acute cellular rejection in liver transplant recipients, which is consistent with other studies.<sup>24</sup> Mean tacrolimus trough levels in patients with acute rejection were 4.05  $\pm$ 1.6 ng/ml as compared to 6.43  $\pm$ 2.2 ng/ml in patients with no rejection. Mean trough levels of tacrolimus in our population without rejection are quite low.<sup>25</sup> Thus, on the basis of our results we can safely recommend tacrolimus trough levels to be in the range of 5 ng/ml to 7 ng/ml to balance risk of acute rejection versus adverse events.

Recent studies have emphasised the importance of unique dosing protocols for tacrolimus-based immuno-

suppression, hinting towards the possibility of genetic polymorphism in drug metabolism across certain patient populations. This study provides data from liver transplant recipients in Pakistan necessitating future molecular and clinical studies to establish national guidelines for effective use of tacrolimus with minimum adverse effects.

## CONCLUSION

Pakistani liver transplant patients show varying pharmacokinetic properties. Lower than expected tacrolimus levels are able to achieve desired results with minimal risk of toxicity. A tacrolimus trough level between 8-12 ng/ml is not required in our population. This has a huge impact considering high cost of the drug and risks of adverse effects with high levels.

## ETHICAL APPROVAL:

Ethical approval was obtained from the Institutional Review Board & Ethics Committee (IRB #: 638-086-2016), Shifa International Hospital Islamabad.

## PATIENTS' CONSENT:

Informed consents were taken from all patients prior to enrollment in the study.

## CONFLICT OF INTEREST:

Authors declared no conflict of interest.

## AUTHORS' CONTRIBUTION:

FA: Conceived and designed study, collected, analyzed and interpreted data; drafted manuscript

MK: Designed study, interpreted data and drafter manuscript, reviewed final draft.

ABH: Designed study, collected, analysed and interpreted data; drafted manuscript, reviewed final draft

FSD: Designed study, interpreted data; drafted manuscript.

AA: Designed study, interpreted data; drafted manuscript.

NJ: Collected and analysed data.

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