



Pharmacokinetics of Tacrolimus in Egyptian Liver Transplant Recipients: Role of the Classic Co-variables

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ABSTRACT

Objectives: This work was performed to study the pharmacokinetics of tacrolimus in adult liver transplant recipients after optimization of all the known classic factors contributing to inter-patient variability in whole blood tacrolimus levels. Also, to detect if any variability in whole blood tacrolimus levels still exists or this variability is only a function of the classic co-variables so that their optimization will diminish or eliminate it. **Methods:** Twenty-Six male patients with end-stage liver disease undergoing living donor liver transplantation were selected from the Gastroenterology Department of the International Medical Center, Cairo, Egypt, were enrolled in the study. A patient is initially considered to be a candidate for this study when tacrolimus was indicated as a part of a triple immune suppressive regimen with mycophenolate mofetil and prednisolone. Patients were selected to have non-significant variations in their demographics and pretreatment clinical data. Blood samples were drawn from each patient before the morning dose at specified intervals and the whole blood was assayed for tacrolimus, using Chemiluminescent Microparticle Immunoassay method (CMIA). Six months after liver transplantation, patients were classified into 3 groups based on their tacrolimus trough levels; normalized by its daily dose (C/D ratio), into fast, intermediate and slow metabolizers. **Results:** The results revealed unpredictable variability in whole blood tacrolimus levels among patients at each sampling time and a marked inter-patient variability in mean whole blood tacrolimus levels among individuals throughout the six months post transplantation period, (P value: <0.0001). Considerable inter-patient variability was also evident in tacrolimus pharmacokinetics. During 1st month post-transplant, tacrolimus C/D ratio varied from 0.53 to 12.2 (ng/ml*1/mg) and tacrolimus oral clearance (CL/F) varied from 3.4 to 79.4 L/hr. At 3rd month post-transplant, tacrolimus C/D ratio varied from 0.78 to 8.50 (ng/ml*1/mg) and tacrolimus CL/F varied from 4.9 to 53.2 L/hr. At 6th month post-transplant, tacrolimus C/D ratio varied from 0.73 to 7.10 (ng/ml*1/mg) and tacrolimus CL/F varied from 5.9 to 56.8 L/hr. The overall mean C/D ratio and oral clearance also showed a great variability among patients with a mean of 2.80±1.89 (CV: 67.5%) and 21.3±12.9 (CV:60.7%), respectively. **Conclusion:** The variability in whole blood tacrolimus concentrations and tacrolimus pharmacokinetics existed in spite of careful patient selection and optimization of all the classic co-variables known to affect tacrolimus concentrations, suggesting the presence of other unstudied factors; the recently evolving genetic factors might contribute to this variability. It is recommended to still considering therapeutic drug monitoring as an integral part of tacrolimus therapy to control variations in response until the discovery of a model that considers all the expected covariates to predict the response.

Keywords: Tacrolimus; C/D ratio; Pharmacokinetics; Liver transplantation; Classic covariables

INTRODUCTION

Tacrolimus has become the most widely used immunosuppressant in living donor liver transplantation (LDLT), as it represents the cornerstone of immunosuppression therapy for solid organ transplant recipients and recently prescribed to nearly 90% of the *de novo* liver transplant recipients¹. Regardless of its success in guaranteeing graft survival, the therapeutic use of tacrolimus is complicated by its narrow therapeutic index, large inter-patient pharmacokinetic (PK) variability and the risk of drug interactions with co-administrated medications².

Overexposure to tacrolimus coincided with toxicity and adverse events as neurotoxicity, nephrotoxicity, gastrointestinal disturbance, type 2 diabetes mellitus and hypertension. Moreover, underexposure may provoke acute rejection incidents³. Thus, it is particularly imperative to develop an individualized immunosuppressive dosage regimen through therapeutic drug monitoring (TDM). Despite the fact that monitoring blood trough concentrations (C_0) is an effective method to adjust the immunosuppressant daily oral doses, clinical studies have discovered that this may not provide an accurate indication of overall drug exposure nor be useful for future dosage prediction among various individuals^{4,5}. Determination of the area under the curve (AUC) over a dosing interval is generally considered the best indicator of tacrolimus exposure; however, this requires gathering of numerous blood samples, which for practical and financial reasons restrict its utilization clinically⁶.

Following oral administration, there is huge variability in the rate of absorption and absolute bioavailability (F), being on average 25–30 % for the immediate-release formulation^{7,8}. In the systemic circulation, tacrolimus binding rate with protein is approximately 99%, primarily binding with α 1-acid glycoprotein and albumin, and it has a high affinity with red blood cells^{7,9}.

A number of factors have been reported to affect the pharmacokinetics of tacrolimus, including time post-transplant, patient demographics (age, race), co-administrated medications (corticosteroids, antifungals, calcium channel blockers, etc.), hepatic and renal functions, donor liver characteristics, food administration, hematocrit levels, and the patient and donor genotypes of metabolic enzymes^{7,10}. However, the pharmacokinetic parameters observed in different races may not be applicable to Egyptian adult liver transplant recipients.

In the current work, we studied the pharmacokinetics of tacrolimus, in liver transplant recipients, after optimization of all the known classic factors contributing to inter-patient variability in tacrolimus whole blood levels, to detect if any variability

in tacrolimus level still exists or this variability is only a function of the classic co-variables so that their optimization will diminish or eliminate it.

PATIENTS AND METHODS

1. Patients

Adult Egyptian patients with end-stage liver disease (ESLD), undergoing living donor liver transplantation (LDLT), were included for the study. They were selected from the Gastroenterology department of the International Medical Center (IMC), Cairo, Egypt, from September 2014 to July 2015. A written informed consent was obtained from each patient. The study protocol was approved by both the ethical committee of the faculty of Pharmacy, Helwan University and the Institutional Review Board of the IMC.

The patient is initially considered to be a candidate for this study when tacrolimus was indicated as a part of a triple immune suppressive regimen. Patients were selected to have non-significant variations in their demographics and pre-transplant clinical data. Full medical history taking, physical examination and complete investigations were obtained upon enrolment into the study. Exclusion criteria were as follows: refusal to sign the informed consent, multi-organ transplantation, concomitant drugs that are known to interact with tacrolimus (e.g. verapamil, diltiazem, phenytoin, fluconazole)¹¹, extra immunosuppressive therapy by Everolimus or Sirolimus, pregnant and breastfeeding women.

2. Study Design

A prospective cohort study was conducted. All included patients were to be followed up for 6 months post transplantation.

3. Treatment Protocol

Based on the hospital protocol, all liver transplant recipients were given 500-1000 mg of methylprednisolone (Solu-Medrol®, Pfizer, Cairo, Egypt) I.V. injection during surgery and initiated a triple immunosuppressive regimen consists of tacrolimus (Prograf®, Astellas Toyoma Co. Ltd-Japan), mycophenolate mofetil (MMF) (Cellcept®, F. Hoffman La Roche, Cairo, Egypt.) and oral corticosteroids (Prednisolone®, Arab Drug Company, Cairo, Egypt) on the first day after the operation.

Tacrolimus was administered orally with initial dose of 0.5 mg twice daily. Patients were given tacrolimus dose at the same time each morning and evening, on an empty stomach. Then subsequent doses were adjusted based on the targeted tacrolimus trough blood concentration within the range of 10-15 ng/ml during the first month after liver transplantation, 8 – 12

ng/ml from 2 to 3 months, and within the range of 5 – 10 ng/ml afterwards to maintain the efficacy and safety of the immunotherapy.

The MMF was administrated orally at a dose of 500-1000 mg daily after meals starting at day 1 postoperative lasting for 3-6 months. An IV methylprednisolone was given at a dose of 80 mg/day then decreased gradually to 20mg/day over the first week of transplantation. Then oral methylprednisolone was given at doses 10-20mg daily for the first 3 months post transplantation.

4. Therapeutic drug monitoring of tacrolimus (TDM)

Whole blood samples for the determination of trough tacrolimus concentrations were taken immediately prior to the morning dose. Tacrolimus trough concentrations were measured three times weekly till the end of the 1st month post-transplantation, once weekly from the 2nd up to the end of the 3rd month, and twice monthly up to the 6th month post-transplantation. Trough levels were measured after 14th day post-transplantation to ensure that tacrolimus reached steady state concentrations, as tacrolimus has a long elimination half-life ($t_{1/2} = 12hr$)¹².

Tacrolimus trough concentrations were determined by the Chemiluminescent Microparticle Immunoassay method (CMIA), using the Abbott Architect[®] analyzer (Abbott Park, IL 60046, USA). The whole blood samples were drawn in EDTA tubes and were frozen at -10 °C or colder. Prior to the initiation of the automated ARCHITECT sequence, the whole blood sample was extracted with a precipitating agent and centrifuged. Then the supernatant was decanted into a transplant pretreatment tube which was placed onto the ARCHITECT iSystem. The sample, assay diluent (MES buffer and sodium chloride) and paramagnetic anti-tacrolimus coated antibody microparticles were combined to create a reaction mixture. The collected samples were assayed, after calibration of the ARCHITECT iSystem apparatus by duplicate determinations of the assay calibrators and construction of a standard curve that was validated and recalibrated by the control results. The calibrators ranged from 0.0 to 30.0 ng/ml. A single sample of each control level was tested to evaluate the assay calibration once every 24 hours each day of use. Tacrolimus concentrations above the assay range i.e. > 30ng/ml, were diluted with 150 μ L (one part) of ARCHITECT Tacrolimus calibrator A, then proceed with the pretreatment procedures and the assay was repeated and the results multiplied by the dilution factor automatically. The CMIA assay measures tacrolimus concentrations in whole blood containing 2-30 ng/ml¹³. Any values reported as <2 ng/ml were excluded from the analysis.

5. Data Collection

The baseline demographic characteristics of recipients including age, gender, indication for transplantation, and Model for End stage Liver Disease (MELD) scores were obtained at the time of transplantation. The calculated MELD score is based on three variables (INR, serum bilirubin and serum creatinine), as it quantifies the severity of liver disease, according to the following formula¹⁴:

$$\begin{aligned} \text{MELD score} &= 10 \times (0.957 \log_e(\text{serum creatinine mg/dl}) \\ &+ 0.378 \log_e(\text{total bilirubin mg/dl}) + 1.120 \log_e(\text{INR}) \\ &+ 0.643) \end{aligned}$$

Moreover, the biochemical tests data including liver function tests [Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), total bilirubin (TBIL), albumin], renal function tests [Serum creatinine (Sr. Creat)], blood urea nitrogen (BUN), Hemoglobin (Hgb), Hematocrit (Hct) and international normalized ratio (INR), were recorded for each patient at the baseline.

6. Pharmacokinetics analysis

Tacrolimus trough concentrations were measured as described in the TDM section and evaluated during the 1st, 3rd and 6th months. The apparent oral clearance and dose normalized concentration were calculated, and the metabolism rate was estimated to be evaluated during the 1st, 3rd and 6th months.

6.1. Apparent oral clearance

Apparent oral clearance (CL/F) was calculated assuming steady state one-compartment model using the following equation¹⁵:

$$CL/F (L/hr) = \frac{\text{Daily Dose (mg)} \times 1000}{C_o (\text{ng/ml}) \times 24}$$

Where, C_o is the trough tacrolimus concentration (ng/ml). Since tacrolimus has a long elimination half-life ($t_{1/2} = 12hr$)¹², steady state trough concentration (14 days post liver transplantation) was used in the previous equation ($C_o \approx C_{ss, av}$)¹⁵.

6.2. Dose normalized concentration ratio (C/D ratio)

To overcome tacrolimus level variation due to doses, tacrolimus dose normalized concentration ratio obtained by dividing the tacrolimus trough concentration by the corresponding daily dose according to the following equation:

$$\begin{aligned} \text{C/D ratio (ng/ml * 1/mg)} &= \frac{\text{blood tacrolimus trough concentration (ng/ml)}}{\text{daily tacrolimus dose (mg)}} \end{aligned}$$

Table 1. Demographics and pre-transplant clinical data of the studied patients (n=26)

Patients' ID	Age (years)	MELD score	Liver function tests					INR (IU)	Renal functions tests		Hematology	
			TBIL (mg/dL)	ALT (IU/L)	AST (IU/L)	T. Protein (g/L)	Albumin (g/dL)		Sr. Creat. (mg/dL)	BUN (mg/dL)	Hgb (g/dL)	HCT (%)
1	47	17	3.4	53	62	6.7	2.6	1.8	0.98	21	10.5	31.5
2	56	16	2.6	41	71	6.8	2.1	2.16	0.78	22	10.5	33
3	46	11	2.4	48	68	6	2.7	1.44	0.75	30	12.5	35.5
4	47	15	3.6	34	41	7.2	2.5	1.7	0.83	35	10.2	32
5	48	15	2.6	37	49	6.2	2.7	1.79	0.84	23	11	33
6	53	13	2.3	48	71	6.9	2.5	1.5	0.85	22	10.6	32.5
7	45	13	3.5	34	68	6.5	2.1	1.53	0.73	24	11.6	33
8	48	14	3.8	36	45	8.2	2.2	1.4	0.88	22	10.2	29
9	51	13	3.4	45	62	5.6	2.9	1.34	0.88	32	12	34
10	52	16	2.9	36	60	6.8	2.6	2.1	0.74	21	12.4	36.1
11	46	17	3.1	35	42	6.5	2.1	1.67	1.06	32	12.1	35.6
12	51	13	3.1	34	55	6	2.4	1.6	0.73	34	12.3	37.5
13	47	14	3.2	35	61	7.3	2.1	1.77	0.7	29	11	31.1
14	58	12	2.8	34	40	6.4	3	1.46	0.8	35	14.4	36
15	55	14	3.7	52	63	6.7	3.1	1.42	0.86	34	10.5	28
16	48	14	2.3	36	38	6	2.9	1.5	1	23	12.6	37.8
17	53	13	2.4	35	42	6.2	2.9	1.5	0.9	24	10.8	31
18	55	14	2.5	34	39	6.7	2.5	1.65	0.89	35	10.5	30
19	56	12	3.2	35	57	6.9	2.1	1.42	0.75	35	11.1	33.5
20	47	9	2.3	53	71	7.2	3.2	1.34	0.7	34	13.1	36.5
21	48	18	3.5	34	48	5.7	2.2	2.3	0.8	24	9.9	27.9
22	51	15	3.8	37	69	8.2	3.1	1.62	0.79	34	10.5	33
23	57	14	2.4	37	68	5.4	2.1	1.8	1.03	34	10.1	28
24	50	21	3.2	43	38	8.3	3	2.41	1.01	33	11.9	35.2
25	52	17	3.1	35	63	6.2	2.1	1.9	0.91	34	9.8	30
26	54	20	3.8	54	68	6.3	3.1	2.1	1.04	35	12	36
Range	45-58	9-21	2.3-3.8	34-54	38-71	5.4-8.3	2.1-3.2	1.34-2.41	0.7-1.06	21-35	9.8-14.4	28-37.8
Mean	51	14	3.03	39.8	56.1	6.65	2.57	1.68	0.86	29.3	11.3	32.9
SD	4	3	0.52	7.1	12.1	0.76	0.39	0.3	0.11	5.6	1.2	2.9
CV %	7.6%	18.2%	17.3%	17.8%	21.6%	11.4%	15.1%	17.9%	12.9%	19.2%	10.1%	9.1%

MELD score: Model for End stage Liver Disease, **TBIL:** total bilirubin, **ALT:** Alkaline Aminotransferase, **AST:** Aspartate Aminotransferase, **T. protein:** total protein, **Sr.creat:** Serum creatinine, **BUN:** Blood urea nitrogen, **Hgb:** Hemoglobin, **HCT:** Hematocrit, **INR:** International Normalized Ratio.

SD: standard deviation, **CV:** coefficient of variation.

6.3. Tacrolimus metabolism rate

At the 6th month post-transplantation, tacrolimus C/D ratio was used as a cut off value for patient categorization regarding tacrolimus metabolism rate. Accordingly, patients were categorized as follows: fast metabolizer for patients with C/D ratio < 1.05 (ng/ml*1/mg), intermediate metabolizers –for patients with C/D ratio of 1.05 – 1.54 (ng/ml * 1/mg) and slow metabolizers for patients with C/D ratio ≥1.55 (ng/ml * 1/mg) ¹⁶⁻¹⁹. Following categorization into fast, intermediate and slow metabolizers, tacrolimus CL/F was compared among these categories.

7. Statistical Analysis

Statistical analyses were performed using IBM SPSS statistical software, version 25 (SPSS Inc, Chicago, IL, USA) and RStudio version 1.1.463 (RStudio Team (2016))²⁰. Descriptive statistics were applied using mean, standard deviation, frequency and percentage. Coefficient of variation was used to test homogeneity. ANOVA test was used for comparison between more than two groups. *P* values < 0.05 were considered statistically significant.

RESULTS

1. Patients' Demographics

A total of 26 adult LDLT male recipients were enrolled into this study. Demographic characteristics and pretreatment clinical data are shown in table (1). There were non-significant variations among patients regarding demographic data and baseline pre-transplant clinical data. Their mean age was 51± 4 years old. All patients suffered from ESLD of chronic liver cirrhosis due to hepatitis C virus (HCV) and treated with LDLT. All Patients had mean MELD score of 14±3 with 18.2% coefficient of variation.

1. Tacrolimus Pharmacokinetics

1.1. Tacrolimus levels

During the follow-up, a total of 750 tacrolimus trough concentrations were collected, with an average of 32 (range 28 - 36) tacrolimus observation were obtained per patient. Variability in tacrolimus trough levels among patients was clearly shown in **Figure 1**.

Results reveled a marked inter-patient variability in the mean C/D ratio among patients during the 1st, 3rd and 6th months post-transplantation. Table (2) clearly presents the high variability in tacrolimus trough concentrations, daily doses and C/D ratios among patients at each time point. At the first month post-transplantation, the mean tacrolimus trough level was 7.2±3.1ng/ml with 43.1% coefficient of variation among patients, while the mean daily dose was 3.6±2.0 mg with 55.6% coefficient of variation among patients, and the

mean C/D ratio was of 2.58±1.99 ng/ml*1/mg with 77.1% coefficient of variation.

Moreover, at 3rd month post-transplantation, the mean tacrolimus trough level was 7.7±3.3 ng/ml with 42.8% coefficient of variation among patients, while the mean daily dose was 3.0±1.6 mg with 53.3% coefficient of variation among patients, and the mean C/D ratio was of 2.92±1.53 ng/ml*1/mg with 52.4% coefficient of variation.

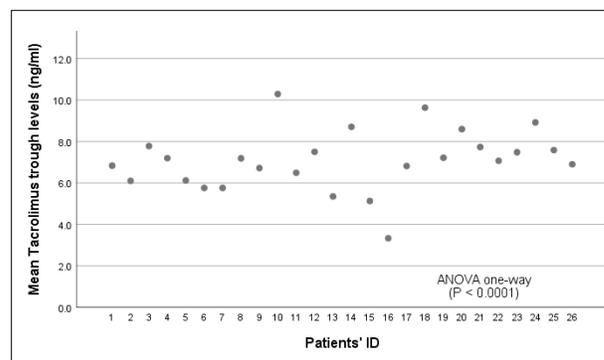


Figure 1. Variation in the mean tacrolimus levels in the studied patients (n=26) during the study period.

Likewise, at 6th month post-transplant, the mean tacrolimus trough level was 6.5±3.4 ng/ml with 52.3% coefficient of variation among patients, while the mean daily dose was 2.1±1.1 mg with 52.4% coefficient of variation among patients, and the mean C/D ratio was of 3.73±2.08 ng/ml*1/mg with 55.8% coefficient of variation.

Also, **Figures 2-4** support the significant variations among the individual patients regarding the mean C/D ratio. At the first month post-transplantation, the mean C/D ratio ranged from the lowest value of 1.08 (ng/ml*1/mg) in patient number 16, to the highest value of 5.48 (ng/ml*1/mg) in patient number 26, with a significant variability between the two patients (*P* value <0.0001). Also, at the 3rd month, the mean C/D ratio ranged from the lowest value of 0.86 (ng/ml * 1/mg) in patient number 25, to the highest value of 5.68 (ng/ml *1/mg) in patient number 4. Moreover, at the 6th month post-transplantation, the mean C/D ratio ranged from the lowest value of 0.75 (ng/ml * 1/mg) in patient number 25, to the highest value of 7.17 (ng/ml * 1/mg) in patient number 18; with evident variability between the two patients (*P* value <0.0001).

1.2. Tacrolimus apparent oral clearance

Inter-patient variability was evident in the calculated tacrolimus apparent oral clearance (CL/F) at the 1st, 3rd and 6th month post-transplantation as shown in **Table 2**.

Table 2. Tacrolimus levels and pharmacokinetics in the studied patients at different post-transplant time periods

Tacrolimus pharmacokinetics	Post-transplant time					
	1 st Month		3 rd Month		6 th Month	
	Mean ± SD	CV%	Mean ± SD	(CV%)	Mean ± SD	(CV%)
Tac C ₀	7.2 ± 3.1 (2.0 – 20.7)	43.1%	7.7 ± 3.3 (2.0 – 19.8)	42.8%	6.5 ± 3.4 (2.1 – 14.4)	52.3%
Daily Dose	3.6 ± 2.0 (1.0 – 11.0)	55.6%	3.0 ± 1.6 (1.0 – 8.0)	53.3%	2.1 ± 1.1 (0.5– 6.0)	52.4%
Tac C ₀ /D ratio	2.58 ± 1.99 (0.53 – 12.2)	77.1%	2.92 ± 1.53 (0.78 – 10.4)	52.4%	3.73 ± 2.08 (0.73 – 7.10)	55.8%
CL/F	23.5 ± 13.4 (3.4 – 79.4)	57.1%	18.2 ± 10.5 (4.0 – 53.2)	57.7%	15.3 ± 9.8 (5.9 – 56.8)	64 %

SD: standard deviation.

CV%: percentage coefficient of variation.

Tac C₀: Tacrolimus trough level (ng/ml).

Tac Daily Dose: Tacrolimus total dose/24 hours in (mg).

Tac C₀/D: Tacrolimus dose normalized concentration (ng/ml * 1/mg).

CL/F: Apparent oral clearance (L/hr).

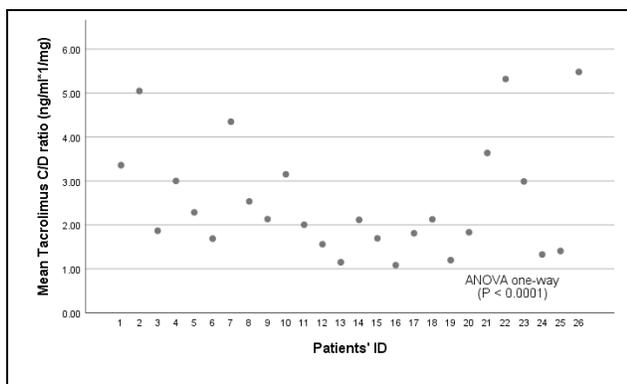


Figure 2. Variation in the mean C/D ratio in the studied patients (n=26) at 1st month post-transplantation.

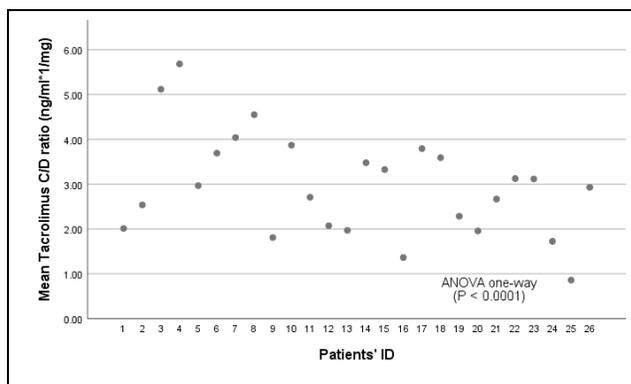


Figure 3. Variation in the mean C/D ratio in the studied patients (n=26) at 3rd month post-transplantation.

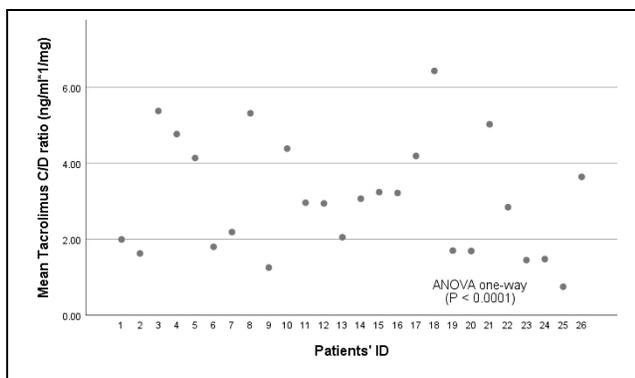


Figure 4. Variation in the mean C/D ratio in the studied patients (n=26) at 6th month post-transplantation.

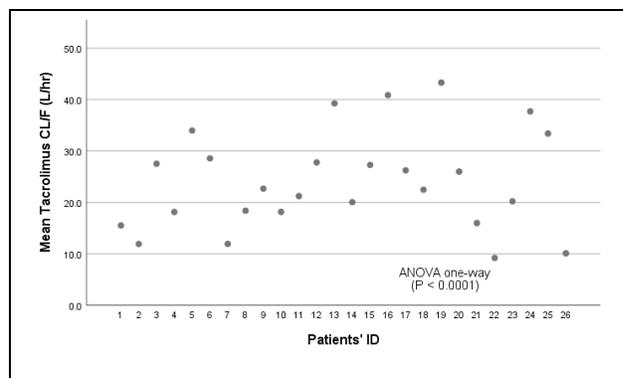


Figure 5. Variation in the mean tacrolimus oral clearance among the studied patients (n=26) at 1st month post-transplantation.

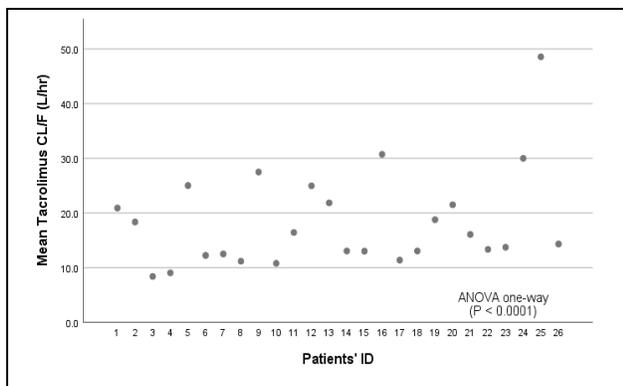


Figure 6. Variation in the mean tacrolimus oral clearance among the studied patients (n=26) at 3rd month post-transplantation.

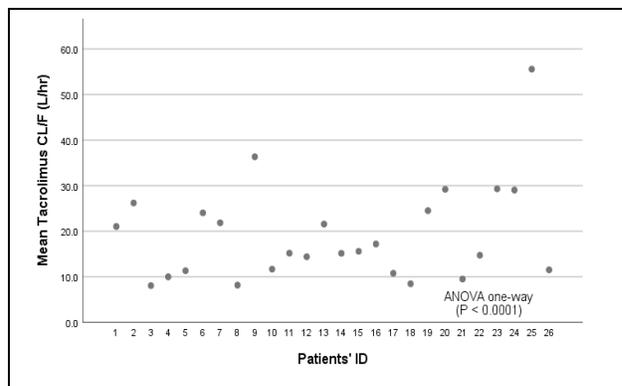


Figure 7. Variation in the mean tacrolimus oral clearance among the studied patients (n=26) at 6th month post-transplantation.

Also, **Figures 5-7** support the significant variations among the individual patients regarding the mean tacrolimus oral clearance. At the 1st month post-transplantation, tacrolimus CL/F ranged from the lowest value of 9.21 L/hr in patient number 22, to the highest value of 43.3 L/hr in patient number 19, with an evident variability between the two patients (P value <0.0001). Also, at the 3rd month post-transplantation, tacrolimus CL/F ranged from the lowest value of 8.4 L/hr in patient number 3, to the highest value of 48.6 L/hr in patient number 25. Likewise, at the 6th month post-transplantation, tacrolimus CL/F ranged from the lowest value of 7.9 L/hr in patient number 18, to the highest value of 55.6 L/hr in patient number 25, with an evident variability between the two patients (P value <0.0001). The overall mean oral clearance showed a great variability among patients, with a mean of 23.5 ± 13.4 L/hr (CV: 57.1%), 18.2 ± 10.5 L/hr (CV: 57.7%), and 15.3 ± 9.8 L/hr (CV: 64%) at the 1st, 3rd and 6th months post-liver transplantation.

1.3. Tacrolimus metabolism rate

Table 3 classifies the patients according to their mean tacrolimus C/D ratio into three groups: fast metabolizers (group I), intermediate metabolizers (group II) and slow metabolizers (group III). From all patients, 3 recipients (11.5%) comprised group I, 7 recipients (26.9%) comprised group II and 16 recipients (61.5%) comprised group III. Also, the calculated mean oral clearance per each group at different time point was determined.

During the 1st month post-transplantation, there were no significant difference among the three groups (P value=0.08), as the mean CL/F was 27.2 ± 10.4 (L/hr), 22.3 ± 12.8 (L/hr), and 23.3 ± 13.9 (L/hr) in the fast, intermediate and slow metabolizers, respectively.

On the contrary, a significant variation was observed in the mean tacrolimus oral clearance among the three groups at 3rd and 6th months with P values of 0.001 and <0.0001, respectively. For the 3rd month post-transplantation, the mean tacrolimus CL/F was 35.2 ± 14.3 (L/hr), 17.9 ± 9.3 (L/hr), and 15.5 ± 5.3 (L/hr) in the fast, intermediate and slow metabolizers, respectively. Furthermore, during the 6th month post-transplantation, the mean tacrolimus CL/F was 39.1 ± 13.9 (L/hr), 20.1 ± 7.9 (L/hr), and 12.3 ± 6.1 (L/hr) in the fast, intermediate and slow metabolizers, respectively.

DISCUSSION

Tacrolimus (also known as FK506), a calcineurin inhibitor (CNI), is the cornerstone in the immunosuppressive regimen post liver transplantation²¹. Regardless of its success in guaranteeing graft survival, therapeutic use of tacrolimus is complicated by its narrow therapeutic index, interpatient pharmacokinetics' variability and the risk of drug interactions with co-administrated medications¹¹. Many factors can cause alteration in tacrolimus pharmacokinetics and hence inter-individual variability in response to tacrolimus therapy is expected and have been extensively reviewed in the literature²². These factors included; age, height, body weight, gender, type of the graft, hepatic function, renal function, hematocrit levels, albumin levels, time after transplant, race, concomitant medications, cytochrome P450 expression, drug formulation, dose given, dosing interval, patient compliance and the assay used in measuring drug concentrations^{17,23-26}. In the current work, we studied the pharmacokinetics of tacrolimus in a relatively homogenous group of LDLT recipients, carefully selected on the basis of having non-significant differences in terms of their demographics

and baseline clinical presentation as well as having all the classic co-variables known to affect tacrolimus concentrations optimized. We aimed at determining if variability in whole blood tacrolimus concentrations still exists in spite of this optimization; suggesting the presence of other unstudied factors or the optimization in patient selection on the above bases would diminish or eliminate variability.

All the study participants were Egyptian patients to avoid any variability in tacrolimus levels due to ethnic factors²⁷. As Felipe et al;²⁸ showed that in order to attain comparable tacrolimus levels, African American patients required higher doses of tacrolimus than Caucasians, due to significant variations in the absolute bioavailability of tacrolimus among different races²⁹. Pediatric and elderly individuals over 60 years were excluded. This exclusion was to avoid variability in tacrolimus pharmacokinetics that might be introduced by age differences^{27,30}. The age-dependent variation of tacrolimus pharmacokinetics⁷ is widely supported in pediatric liver recipients. Actually, in order to preserve similar tacrolimus levels, pediatric transplant patients demand 2-4-fold adult tacrolimus doses, this could be explained by the metabolic function of the liver during the child's developmental stage³¹. Additionally, David et. al,³² conducted a subgroup analysis of the pharmacokinetics of tacrolimus in 44 elderly recipients (65±3 years) compared to 31 younger controls (35±6 years), and demonstrated significant lower total body clearance of tacrolimus in the elderly group (p<0.0001). Subjects in our study were chosen to have close ages (45-58), in order to avoid any age related physiological changes that might alter tacrolimus pharmacokinetics^{23,24,27}. The study design also, excluded women to avoid variations related to gender. As previously reported by Stratta et. al³³ that a higher tacrolimus clearance was observed in female patients compared to male patients.

Some disease states may influence tacrolimus pharmacokinetics and dosages changes are then required. It was previously reported by Trotter et. al,³⁴ that HCV patients are expected to receive reduced mean tacrolimus doses to achieve targeted tacrolimus trough concentrations. It was suggested that hepatitis virus replication in liver cells change the CYP system and thus reduces the metabolism of tacrolimus. Regarding our cohort study, patients suffered from ESLD that required LDLT due to chronic liver cirrhosis with HCV were only included, in order to eliminate inter-individual variability of tacrolimus that might be related to other indication for liver transplantation, as well as all patients had similar MELD score with a mean value of 14±3 and a coefficient of variation of 18.2%.

Clinically significant variability in tacrolimus bioavailability and its dependence on dosage form has been recognized as a major medical concern. Recently, numbers of published studies reported the inter-subject

variability in tacrolimus bioavailability as a function of different dosage forms^{35,36}. Participants in the present study were given the same drug formulation through the same route of administration; they all received oral tacrolimus in the form of immediate release Prograf®, (Astellas Toyoma Co. Ltd-Japan.), 0.5 mg oral-capsules twice daily.

Blood samples were appropriately drawn, all were analyzed using the same method and the same laboratory, in order to avoid any variation in the precision and accuracy of the results obtained¹³. Our study design selected a chemiluminescent microparticle immunoassay (CMIA) tacrolimus assay on the Abbott Architect® analyzer, based on the study conducted by Bazin et al¹³ who reported that the tacrolimus CMIA assay serves as a valuable screen for tacrolimus pharmacokinetics.

In blood, tacrolimus is strongly bound to erythrocytes and plasma proteins, and their concentrations is greatly affected by hypoalbuminemia and anemia³⁴. Minematsu et. al;³⁴ have examined the effect of hematocrit on tacrolimus pharmacokinetics in adult LDLT recipients, and concluded that patients with low hematocrit levels were associated with an increase in the tacrolimus oral clearance. Although, the fact that the patients in our study were diagnosed with end stage liver disease and subjected to liver transplantation, they were carefully selected on a basis of having homogenous range of hematocrit and albumin levels with coefficient of variation of (9.07%) and (15.07%), respectively.

Pharmacokinetics of tacrolimus have been intensively reviewed in the literature and considerable data have been published regarding tacrolimus C/D ratio and steady-state pharmacokinetics^{16,19,37}. Data regarding steady-state tacrolimus pharmacokinetics in the adult Egyptian liver transplant recipients are not available. The current study presented greatly significantly varied tacrolimus steady-state pharmacokinetics among the selected Egyptian liver transplant patients, with coefficients of variation of major differences, suggesting significant inter-individual variability.

In spite of all our efforts to optimize and reduce the influence of the classic factors known to affect tacrolimus C/D ratio and hence patients' response to therapy, and in spite of individualizing tacrolimus regimens to targeted blood concentrations, a significant inter-patient variability in mean tacrolimus C/D ratio over the 6 months of follow up was clearly apparent (P value: <0.0001).

Since tacrolimus whole blood trough levels and their corresponding doses were routinely recorded for therapeutic drug monitoring of tacrolimus, calculating the C/D ratio could be used as an established tool to determine the tacrolimus metabolism rate^{17,19}. Because of the possible interaction between tacrolimus and high

Table 3. Patients' classification according to the metabolism rate of tacrolimus and their mean oral clearance at different post-transplant time periods

During the 1 st month	No. of patients	CL/F (L/hr) Mean ± SD (Range)	P value
Group I: Fast metabolizers	3 patients	27.2 ± 10.4 (9.4 – 59.5)	0.08
Group II: Intermediate metabolizers	7 patients	22.3 ± 12.8 (3.4 – 66.7)	
Group III: Slow metabolizers	16 patients	23.3 ± 13.9 (3.5 – 79.4)	
During the 3 rd month	No. of patients	CL/F (L/hr) Mean ± SD	P value
Group I: Fast metabolizers	3 patients	35.2 ± 14.3 (13.6 – 53.2)	0.001
Group II: Intermediate metabolizers	7 patients	17.9 ± 9.3 (4.1 – 50.5)	
Group III: Slow metabolizers	16 patients	15.1 ± 5.3 (5.9 – 29.8)	
During the 6 th month	No. of patients	CL/F (L/hr) Mean ± SD	P value
Group I: Fast metabolizers	3 patients	39.1 ± 13.9 (17.7 – 56.8)	<0.0001
Group II: Intermediate metabolizers	7 patients	20.1 ± 7.9 (8.1 – 33.3)	
Group III: Slow metabolizers	16 patients	11.8 ± 5.9 (5.9 – 36.2)	

SD: standard deviation.

P value calculated by ANOVA one-way statistical analysis.

The patient is considered a fast metabolizer when its C/D ratio <1.05 (ng/ml*1/mg), an intermediate metabolizer when its C/D ratio = 1.05-1.54 (ng/ml*1/mg), and a slow metabolizer when its C/D ratio ≥1.55 (ng/ml*1/mg).

corticosteroid doses^{38,39}, the C/D ratio, in the present study, was assessed 6 months post liver transplantation to determine the tacrolimus metabolism rate at which corticosteroid was discontinued. The study classified the patients into 3 groups according to their tacrolimus metabolism rate as defined previously^{16,19}. A significant difference in mean oral clearance was observed among the three groups (fast, intermediate and slow metabolizers) at 3rd and 6th months post-transplantation (P value: 0.001 and <0.0001, respectively), while there was no significant difference in mean CL/F during the 1st month post-transplantation. This could be explained by the concomitant high corticosteroid therapy early post-transplantation that induces CYP3A5 enzymes and therefore increase mean tacrolimus oral clearance during the 1st month post-transplantation, while the mean tacrolimus CL/F decreases at 3rd and 6th months upon discontinuation of the oral corticosteroid therapy^{40,41}. Activity of the microsomal enzymes varies greatly among individuals and hence their activation would be greatly varied accordingly⁴². This may explain, in part, the increased variability of the tacrolimus CL/F due to concomitant corticosteroids administration, during the 1st

month and hence the insignificant difference among the tested categories.

Our results showed great variability in tacrolimus apparent oral clearance among the studied population (CV: 60.82%). Furthermore, the mean tacrolimus CL/F varied during the 1st, 3rd, and 6th month post-transplantation was 23.5±13.4 (L/hr), 18.2±10.5 (L/hr) and 15.3±9.8 (L/hr), respectively. Our results are comparable with Schafer et.al⁴³, as they reported the mean of tacrolimus relative clearance was 20.7(l/hr), 19.5 (l/hr) and 15.9 (l/hr) at the 1st, 3rd and 6th months post-transplant, respectively.

Variability in whole blood tacrolimus levels at the trough concentration is probably attributed to variability in absorption as well as variability in elimination. However, since tacrolimus is mainly metabolized in the liver and primarily excreted through bile, and its renal clearance only accounted for ≤ 1.1% of the total tacrolimus clearance⁵, and since all patients had comparable liver and kidney functions pre-transplant, we therefore hypothesize that, this variability in whole blood tacrolimus levels might be attributed to, variability in the expression levels of the CYP3A5 and P-glycoprotein⁴⁴.

Passey et al,⁴⁵ proposed that the genetic variables could contribute considerably to the pharmacokinetic variability of tacrolimus in addition to the various covariates that trigger drug-response variability among patients. Indeed, recent studies have reported that much of the inter-patient variation associated with tacrolimus dose requirement to attain the target blood levels mainly due to difference in CYP4A5 gene polymorphism. In 2013, Rojas et al⁴⁶ conducted a meta-analysis and reported that CYP3A5 gene polymorphisms affects the tacrolimus C/D ratio. Wild-type allele (CYP3A5*1) individuals have the largest quantity of CYP3A5 protein in both their liver and small intestine, therefore, higher tacrolimus doses were required to attain the targeted tacrolimus blood levels. Meanwhile, a marked decline in CYP3A5 expression associated with SNPs within CYP3A5 (e.g. CYP3A5*4)⁴⁷.

The above findings confirm the presence of considerable inter-subject variation in tacrolimus pharmacokinetics even after ensuring close matching of all individual characteristics and study parameters. The first step in minimizing drug-response variability among individuals is always to define its mechanism by which the variability might occur. Since we optimized all factors known to contribute to the individual variability in tacrolimus pharmacokinetics and patients' response, and since this variability is still clearly obvious, presence of unstudied factor(s), that might play a role in this great variability, must be suggested.

CONCLUSIONS AND RECOMMENDATIONS

The variability in whole blood tacrolimus concentrations and tacrolimus pharmacokinetics existed despite of careful patient selection and optimization of all the classic co-variables known to affect tacrolimus concentrations, suggesting the presence of other unstudied factors; the recently evolving genetic factors might contribute to this variability. It is recommended to still considering therapeutic drug monitoring as an integral part of tacrolimus therapy to control variations in response until the discovery of a model that considers all the expected covariates to predict the response.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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