ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 7, 2025

Online - 2455-3891
Print - 0974-2441

Research Article

# CORRELATION OF CLINICAL LABORATORY PARAMETERS AND DEMOGRAPHY WITH PHARMACOKINETICS OF TACROLIMUS – A NARROW THERAPEUTIC INDEX DRUG

**SAJAD KHALIQ DAR[1,2], ARSHAD H KHUROO[1], MOHD AKHTAR[2]*, SUDERSHAN KUMAR[1]**

[1]Department of Clinical Pharmacology and Pharmacokinetics, Sun Pharmaceutical Industries Limited, Gurugram Haryana, India.
[2]Department of Pharmacology, School of Pharmaceutical Education and Research, Jamia Hamdard (Hamdard University), New Delhi, India.
*Corresponding author: Mohd Akhtar; Email: makhtar_ph@jamiahamdard.ac.in

*Received: 08 April 2025, Revised and Accepted: 20 May 2015*

**ABSTRACT**

**Objectives:** During clinical pharmacokinetic (PK) studies, human volunteers are subjected to screening with respect to clinical pathology parameters, based on which volunteers are considered healthy or not healthy. Even for the screened healthy volunteers, these parameters vary from person to person, and the reason is obvious, these parameters fall in a well-defined acceptable range. The diversity in the physiological and pathological values, even within the acceptable limits, could be significant enough to have an impact on systemic drug exposure. The present research was aimed to study the correlation between the clinical parameters (used for screening) and PKs of tacrolimus, which is a narrow therapeutic index (NTI) drug.

**Methods:** Twenty-four healthy adult human subjects, aged between 18 and 50 years, were recruited in this single-dose, open-label, balanced, randomized crossover study under fasting conditions. The eligibility of volunteers was based on inclusion and exclusion criteria mentioned in the study protocol, and screened healthy volunteers were enrolled in the study. Dose administration was done after fasting of 10 h and blood samples were collected for measurement of the tacrolimus concentrations by a validated liquid chromatography-mass spectrometry method. PK parameters were calculated followed by their correlation analysis with respective clinical pathological/screening parameters.

**Results:** The results of this study suggest that values of clinical parameters can increase or decrease the systemic exposure of the tacrolimus. A significant correlation was observed between PK estimates and age, serum creatinine levels, hematology, and total bilirubin. The coefficient of correlation was approximately 0.9.

**Conclusion:** The information on the correlation of demography, hematology, serology, and biochemistry with PK would assist PK researchers in having an optimum study design for the successful conduct of clinical studies, especially for NTI, cytotoxic, or drug molecules with high PK variability. These studies will also support clinicians to devise an effective drug therapy, especially for NTI medications intended for long-term treatment.

**Keywords:** Pharmacokinetics, Groups, Significant, Phoenix, SAS, Hemoglobin, Creatinine.

 DOI: http://dx.doi.org/10.22159/ajpcr.2025v18i7.54513. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

## INTRODUCTION

Bioequivalence (BE) studies are critical in the new drug development process and are required for the approval and marketing of generic drug products. These studies are generally aimed to determine that there is no significant difference between two drug products in terms of the rate and extent to which the active drug ingredient or active moiety becomes available at the site of drug action [1]. According to the criteria developed by various regulatory agencies, especially the U.S. Food and Drug Administration, two pharmaceutically equivalent products are bioequivalent when the 90% confidence interval of the geometric mean ratio area under the curve (AUC) and $C_{max}$ fall within 80–125% [2]. This approach thus indicates the 20% difference between the two drug products is clinically not significant. For some drugs where small differences in dose or blood concentration could produce serious therapeutic failures and/or adverse drug reactions, a 20% difference in blood concentration or drug exposure may be unacceptable. Such drugs are narrow therapeutic index (NTI) drugs [3]. Regulatory agencies, such as Health Canada and the European Medicines Agency, tightened the average BE limits for NTI drugs in concurrence with the meeting by the Advisory Committee for Pharmaceutical Science and Clinical Pharmacology in April 2010. The meeting stated that the requirements for confidence intervals should be narrower (90–111%) and "Replicate studies are important" for such drugs [4-7].

Tacrolimus, an immunosuppressant commonly used to prevent and treat allograft rejection in transplant recipients, belongs to the NTI category [8,9]. Like other NTI drugs, treatment with tacrolimus must be monitored closely to maintain drug concentrations within the therapeutic window. The systemic concentrations, if too high, can cause serious adverse effects and, if too low, can result in transplant rejection [9]. The NTI of tacrolimus limits its use and requires therapeutic drug monitoring (TDM) to optimize the dose to prevent toxicity and rejection [9,10]. Further, there is a significant correlation between its serum or dose level and the deterioration of the glomerular filtration rate (GFR) and serum creatinine in transplanted patients [11]. The clearance (CL) of tacrolimus is influenced by multiple factors which include cytochrome P450 (CYP) 3A genotype [12,13], age, body weight, and ethnicity [14]. Therefore, dose adjustment through TDM becomes very important to limit the duration for which a patient is exposed to concentrations outside the target range [10]. In addition to all the challenges associated with tacrolimus, it shows significant pharmacokinetic (PK) variability due to which the dose required to achieve target concentration varies from person to person and within an individual [15].

Based on these reasons, the conduct of BE studies for NTI drugs such as tacrolimus comes with multiple challenges. The common factors such as sample size, study design, and within-subject variability have been addressed through various research to have an impact on the study outcome. These factors are crucial, but other factors, such as

physiological or pathological variability of the subjects, which could affect the PK values (drug concentration) directly or indirectly, cannot be ignored. During clinical studies, the volunteers are screened, and these screening parameters or the inclusion criteria may vary from one subject to another. The diversity in the physiological and pathological values, even within the acceptable limits, could be significant enough to impact systemic drug exposure.

This research was aimed to study the correlation between clinical screening parameters such as routine serology, hematology, and demography used for the enrolment of study volunteers with final PK point estimates.

## METHODS

### Participants and study design

This was a single-dose, open-label, balanced, and randomized crossover fasting study. The study protocol was approved by the Jamia Hamdard Institutional Ethics Committee in New Delhi. Written informed consent was received from all the study subjects before participation in the study. Twenty-four healthy adult human subjects, aged between 18 and 50 years, were recruited in the study. Inclusion criteria were (i) absence of acute or chronic diseases that could affect vital organ functions, (ii) no history of surgery within the past 6 months, (ii) no history of hypersensitivity reactions or idiosyncratic reactions to tacrolimus, (iii) no history or current drug abuse, (iv) ability to communicate (reading, writing, and speaking) effectively, and (v) willingness to give informed consent for study participation. Exclusion criteria were (i) clinically significant abnormality during physical examination, (ii) clinically significant abnormality of electrocardiograms (ECGs) or chest X-ray, (iii) pregnancy or lactation, (iv) blood tests positive for hepatitis B surface antigen, hepatitis C virus, or human immunodeficiency viruses, (v) abnormality in blood coagulation or history or concurrent use of anticoagulants or antiplatelets, or (vi) participation in any other study in the past 3 months.

### Screening of study subjects

After obtaining the written informed consent, clinical and laboratory investigations were carried out to confirm the eligibility of the research participants. These included physical examination, ECG monitoring, chest X-ray test, and laboratory investigations (hematology, serum biochemistry, urinalysis, serology, and pregnancy status). Recruitment continued till the required number of eligible study participants was achieved. The study was designed to have both male and female volunteers; however, only male volunteers were recruited due to the unavailability of desired female volunteers. Medically healthy subjects with clinically acceptable laboratory profiles screened within 28 days before initiation of the study were enrolled in the study.

### Randomization

A randomization schedule for the administration of investigational products to each subject was generated using SAS software. The design of the study was three sequences and three periods wherein each subject was randomly assigned to one of the three treatments in the first period and other treatments in the following periods.

### Drug administration

All the recruited subjects were admitted to the clinical pharmacology unit well before time to meet the 10 h fasting condition before dosing. Twenty-four subjects were dosed with a single oral dose of either one tablet of Test products (two prototypes) or Reference product with 240 mL of drinking water at ambient temperature after at least 10 h of fasting during each period of the study. Water was restricted for 1 h before and after dosing, except for 240 mL of water given at the time of drug administration. All volunteers had to continue the fast till 4 h post-dose.

### PK evaluation

*Blood sample collection*

Venous blood samples were collected through an indwelling intravenous cannula inserted in the forearm of the subjects during 24 h period of frequent blood sampling. The patency of the cannula was maintained with 5 IU/mL of heparin in a normal saline solution. Blood samples were collected in pre-chilled $K_3$EDTA vacutainers before drug administration (pre-dose) and at 0.500, 1.000, 1.500, 2.000, 3.000, 4.000, 5.000, 6.000, 7.000, 8.000, 9.000, 10.000, 11.000, 12.000, 14.000, 16.000, 20.000, 24.000, 36.000, 48.000, 72.000, 96.000, 120.000, and 144.000 h after dose administration. Blood samples were kept in an ice cold water bath till storage in freezers at or below -15°C before analysis.

### Determination of tacrolimus concentration

Tacrolimus was extracted from the human blood using a solid phase extraction technique. Chromatographic separation using the Shimadzu Nexera X2 high-performance liquid chromatography system (Shimadzu Corporation; Kyoto, Japan) was achieved on a reversed-phase C18 analytical column (ACE 3 C18-PFP [150 mm × 4.6 mm, 3 µm]). An isocratic solvent system of 5 mm ammonium acetate and acetonitrile 10:90 with 0.1% formic acid v/v at a flow rate of 0.7 mL/min was run for a total run time of 5 min. A triple quadrupole mass spectrometer, MDS Sciex API-4000 (MDS Sciex, Toronto, Ontario, Canada), equipped with electrospray ionization (LC-ESI-MS/MS) was tuned for detection in positive ionization mode. The *m/z* transitions observed in multiple reaction monitoring modes were *m/z* 821.6 → 768.4 and *m/z* 809.6 → 756.4 for tacrolimus and tacrolimus-related compound A (internal standard), respectively. Analysis for tacrolimus was performed on whole blood samples, and the linear concentration range used was 0.2–50 ng/mL. The analysis included blood samples from the subjects who completed at least two periods of the study.

### PK analysis

The PK parameters were estimated from the blood concentrations obtained from study subjects by the non-compartmental model using Phoenix® WinNonlin® 8.1 Pharsight, Inc. USA. Scheduled sample collection time was used for PK analysis. Data from volunteers who had completed at least two periods of the study was used for PK analysis. The completion of the reference treatment period was necessary for the inclusion of data for PK analysis. Twenty-three out of 24 enrolled volunteers completed the reference treatment. The maximum concentration observed (referred to as $C_{max}$) and the time at which maximum concentrations occurred (referred to as $T_{max}$) were obtained directly from the blood concentration-time data. Terminal elimination half-life ($t_{1/2}$) was calculated using the blood concentration-time data, and the AUC from zero time to the last observed time ($AUC_{0-t}$) was calculated using the linear trapezoidal rule. The PK parameters $C_{max}$, $AUC_{0-t}$, $AUC_{0-\infty}$, $T_{max}$, $k_{el,}$ and $t_{1/2}$ were determined from blood concentrations.

### Statistical analysis

Statistical analysis was performed using the SAS® system for Windows, release 9.4 (SAS Institute Inc., USA). The ratios of test and reference for $C_{max}$ and AUC, along with the confidence interval, were calculated to compare the bioavailability.

### Correlations

Correlation analysis was performed for PK point estimates with clinical pathology parameters (hematology, serology, and biochemistry) and demographic values. The PK values of the reference product, which was a marketed product were alone used for correlation analysis. The correlation analysis was performed between PKs and demography (age and body mass index [BMI]), blood parameters (hemoglobin [Hb], total red blood cell [TRBC], and hematocrit [HCT]), kidney parameters (serum creatinine) and liver parameters (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]). The data were divided into multiple groups based on the observed values for each clinical parameter, and the mean value was calculated for each group. The mean values of each group for clinical parameters were used for correlation analysis with the average of each respective PK estimate.

## RESULTS

### Demographics and laboratory parameters

The summary of major screening parameters (demography, hematology, serology, etc.), along with their average values used for correlation analysis, is presented in Table 1. The enrolled subjects were between 19

**Table 1: Summary of screening parameters of enrolled subjects used for correlation**

| Subject | Age (year) | BMI ($kg/m^2$) | Hemoglobin (g/dL) | Total RBC count/L | HCT (%) | Creatinine (mg/dL) | Total bilirubin mg/dL | AST (SGOT) (units/L) | ALT (SGPT) (units/L) |
|---|---|---|---|---|---|---|---|---|---|
| N | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 |
| Mean (±SD) | 30.9 (±7.5) | 22.8 (±2.9) | 14.7 (±1.1) | 4.9 (±0.6) | 43 (±2.9) | 0.8 (±0.1) | 0.7 (±0.4) | 27.6 (±7.5) | 28.3 (±12.7) |
| Min | 19 | 19.2 | 11.6 | 3.3 | 34.5 | 0.6 | 0.6 | 14.9 | 14.7 |
| Max | 48 | 29.5 | 16.3 | 6 | 47.9 | 1.1 | 1.4 | 42.2 | 60.3 |

BMI: Body mass index, RBC: Red blood cell, HCT: hematocrit, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, SD: Standard deviation, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase

and 48 years of age, with a mean age of 30.9 years. The data presented in the table shows that all the participants were healthy; however, the variation for each parameter between the subjects was higher.

**PK and statistics**

The PK parameters calculated using non-compartmental analysis and used for correlation analysis are presented in Table 2. A total of 23 subjects completed the reference treatment, and the summary of the estimated PK parameters is in Table 2. $C_{max}$ values were in the range of 8.80 ng/mL to 36.89 ng/mL, $AUC_{0-t}$ values in the range of 337.596 hr*ng/mL to 973.528 hr*ng/mL, and $AUC_{0-\infty}$ values in the range of 363.756 hr*ng/mL to 1026.837 hr*ng/mL. The difference between the minimum and the maximum values observed for both $C_{max}$ and AUC were significant even for the same formulation, which proves the high variability of tacrolimus. The bioavailability of the test product was toward the lower side compared to the reference product, and due to high intra-subject variability, the difference between the lower confidence interval and upper confidence interval was significant.

**Correlations**

The subjects were divided into multiple age groups (Table 3), and a significant correlation was observed between the age and the PK values. The lowest average values for $C_{max}$ and AUC were observed in the age group 18–24 (lowest age group) and maximum in the age group 40–50 (highest study age group). In addition to $C_{max}$ and AUC values, the observed half-life was also comparatively higher for upper age groups.

The data presented in Table 4 again shows significant correlations between serum creatinine levels and PK values. Serum creatinine levels of 0.6–0.72 (average 0.69) show lower concentrations of $C_{max}$ and AUC with observed half-life also toward the lower side. This indicates comparatively faster elimination and lower accumulation of the drug in the systemic circulation. On the other hand, serum creatinine levels >0.8 displayed higher concentrations of $C_{max}$ and AUC with observed half-life toward the higher side, indicating comparatively slower elimination of the drug.

Hematology parameters such as Hb, HCT, and TRBC have also shown a positive correlation with the PK of tacrolimus. Higher drug exposure ($C_{max}$ and AUC) was observed at upper hematology values (Tables 5-7).

The correlation of total bilirubin count with the PK of tacrolimus displayed a similar trend as observed for the above-mentioned parameters (Table 8).

The coefficient of correlations for age, serum creatinine, and total bilirubin with PK estimates was around 0.9 (Table 9). The parameters such as BMI, ALT, and AST did not show any trend or correlation with the PK point estimates, which could be due to the inclusion of a controlled population and a smaller sample size.

**DISCUSSION**

Clinical investigations such as hematology, serology, and biochemistry, along with demography, are very important parameters to consider before exposing a body to drug therapy, especially for NTI drugs. These clinical parameters determine the overall healthy/unhealthy status of subjects/patients who are to be enrolled in the clinical studies.

**Table 2: Summary of PK parameters of enrolled subjects used for correlation**

| Subject | $C_{max}$ (ng/mL) | $AUC_{0-t}$ (hr*ng/mL) | $AUC_{0-\infty}$ (hr*ng/mL) |
|---|---|---|---|
| N | 23 | 23 | 23 |
| Mean (±SD) | 17.23 (±7.57) | 337.596 (±208.755) | 363.756 (±225.129) |
| Min | 8.80 | 117.822 | 134.136 |
| Max | 36.89 | 973.528 | 1026.837 |

AUC: Area under the curve, SD: Standard deviation, PK: Pharmacokinetics

A clinician or a PK scientist will have to rely on the acceptable range for inclusion of patients/subjects in a study, and the difference in clinical parameters (within an acceptable range) may have a potential impact on the concentration levels, which could impact PK point estimates, especially for NTI drugs such as tacrolimus.

The correlation analysis suggests increased drug systemic levels with increasing age. The aging process can cause changes in body composition and hepatic and renal function, which are responsible for an increase in the volume of distribution and reduced CL of drugs. These changes can lead to a prolongation of plasma elimination half-life, causing significant pharmacodynamic changes, which, in general, tend to increase sensitivity to drugs [16]. An age-dependent decline in total CL can be expected for all drugs that are mostly eliminated by the kidneys, and the reduction in drug elimination by the kidneys in elderly people will result in increased systemic drug levels [17]. Furthermore, the water content of the body decreases, and fat content rises with age, which leads to a reduction in the distribution volume of hydrophilic compounds and an increase in lipophilic drugs. However, intestinal absorption is not affected for most of the drugs, due to which the systemic exposure will be high for such drugs in elderly patients [18]. The comparatively higher systemic drug levels of tacrolimus observed in the current study for upper age groups could be a typical example of age-related changes in metabolism and other physiological functions. Further, with increasing age, the chances of comorbidity and polypharmacy are higher, and these conditions, together with age-related changes in PK and pharmacodynamics, make elderly patients vulnerable to adverse drug reactions, leading to relevant health burdens and costs [19].

Another highlight of the present study was higher systemic exposure for volunteers with comparatively higher serum creatinine levels. The correlation between serum creatinine and drug PK values is obvious, as serum creatinine values indicate kidney functioning. The decreased kidney functioning means less elimination of the drug and the corresponding increase in systemic exposure [18]. Therefore, serum creatinine values or kidney functioning is an important factor for the selection of dose especially for drugs such as tacrolimus. The error in the dosing of NTI drugs or even non-NTI drugs in patients with renal impairment can cause adverse effects and poor outcomes [20,21]. Furthermore, patients with higher BMI tend to have an increased GFR, which can be a sign of kidney damage [22]. Hence, dose selection in such scenarios becomes challenging for clinicians. The serum creatinine or renal function can normally be correlated with age and both these parameters alone as well as together affect the PK of the drugs.

**Table 3: Summary of age groups and their respective mean pharmacokinetic values**

| Age group (Years) | $C_{max}$ (ng/mL)# | $AUC_{0-t}$ (hr*ng/mL)# | $AUC_{0-\infty}$ (hr*ng/mL)# | T½ (hr)# |
|---|---|---|---|---|
| 18–25 (n=5) | 13.885 (±4.485) | 305.745 (±190.129) | 320.386 (±196.153) | 32.942 (±5.030) |
| 26–33 (n=6) | 12.946 (±2.772) | 285.337 (±119.906) | 315.030 (±152.407) | 36.734 (±11.911) |
| 34–40 (n=6) | 17.627 (±5.386) | 319.289 (±210.549) | 349.919 (±237.368) | 39.705 (±7.636) |
| 40–50 (n=6) | 23.905 (±10.596) | 434.703 (±296.831) | 462.462 (±309.844) | 40.506 (±8.440) |

#Mean (±SD). AUC: Area under the curve, SD: Standard deviation

**Table 4: Summary of mean serum creatinine concentrations and their respective mean pharmacokinetic values**

| Serum, creatinine (mg/dL) Group | $C_{max}$ (ng/mL)# | $AUC_{0-t}$ (hr*ng/mL)# | $AUC_{0-\infty}$ (hr*ng/mL)# | T½ (hr)# |
|---|---|---|---|---|
| 0.63–0.74 (n=7) | 14.601 (±5.258) | 278.757 (±124.003) | 294.490 (±128.824) | 32.744 (±6.985) |
| 0.75–0.84 (n=8) | 14.158 (±5.106) | 286.134 (±174.340) | 312.464 (±197.332) | 33.842 (±10.398) |
| 0.85–1.1 (n=8) | 22.603 (±8.916) | 440.542 (±273.471) | 475.657 (±289.846) | 36.804 (±10.986) |

#Mean (±SD). AUC: Area under the curve, SD: Standard deviation

**Table 5: Summary of mean hemoglobin concentrations of different groups and their respective mean pharmacokinetic values**

| Hemoglobin# (Group) | $C_{max}$ (ng/mL)# | $AUC_{0-t}$ (hr*ng/mL)# | $AUC_{0-\infty}$ (hr*ng/mL)# | T½ (hr)# |
|---|---|---|---|---|
| 12.9(±0.957) (Group- 1) | 14.980 (±6.096) | 312.865 (±143.137) | 334.788 (±145.017) | 27.787 (±9.035) |
| 14.5 (±0.206) (Group-2) | 18.005 (±7.466) | 326.060 (±172.194) | 352.863 (±189.107) | 35.685 (±10.662) |
| 15.5 (±0.276) (Group-3) | 14.383 (±5.328) | 276.730 (±177.072) | 300.596 (±202.374) | 35.741 (±8.983) |
| 16.3(±0.1291) (Group-4) | 29.632 (±6.148) | 682.430 (±232.067) | 723.354 (±249.249) | 38.067 (±2.110) |

#Mean(±SD), Group-1&4; n=4, Group-2; n=8 & Group-3; n=7. AUC: Area under the curve, SD: Standard deviation

**Table 6: Summary of mean TRBC count and their respective mean pharmacokinetic values**

| TRBC Count Group | $C_{max}$ (ng/mL)# | $AUC_{0-t}$ (hr*ng/mL)# | $AUC_{0-\infty}$ (hr*ng/mL)# | T½ (hr)# |
|---|---|---|---|---|
| 3.3–4.4 (n=6) | 12.725 (±2.707) | 264.398 (±127.527) | 295.961 (±160.913) | 33.967 (±15.456) |
| 4.5–5 (n=7) | 16.777 (±5.417) | 315.348 (±186.075) | 335.428 (±191.167) | 32.628 (±6.285) |
| 5–5.5 (n=6) | 20.092 (±11.151) | 393.512 (±271.302) | 414.297 (±286.174) | 33.849 (±4.757) |
| Above 5.5 (n=4) | 20.621 (±6.552) | 405.431 (±270.945) | 447.520 (±305.435) | 41.744 (±10.201) |

#Mean (±SD). AUC: Area under the curve, SD: Standard deviation, TRBC: Total red blood cell

**Table 7: Summary of mean Hematocrit concentrations and their respective mean pharmacokinetic values**

| HCT (%) Group | $C_{max}$ (ng/mL)# | $AUC_{0-t}$ (hr*ng/mL)# | $AUC_{0-\infty}$ (hr*ng/mL)# | T½ (hr)# |
|---|---|---|---|---|
| ≤40 (n=5) | 14.370 (±5.452) | 290.303 (±133.833) | 312.773 (±134.892) | 28.866 (±8.188) |
| 41–42 (n=5) | 15.316 (±2.928) | 345.053 (±216.854) | 381.980 (±238.989) | 41.794 (±11.600) |
| 42–44 (n=8) | 18.161 (±9.261) | 322.764 (±177.931) | 345.462 (±202.272) | 32.227 (±9.177) |
| Above 44 (n=5) | 20.515 (±9.825) | 401.163 (±333.144) | 425.788 (±350.290) | 36.653 (±4.381) |

#Mean (±SD). HCT: Hematocrit, AUC: Area under the curve, SD: Standard deviation

**Table 8: Summary of mean total bilirubin concentrations and their respective mean pharmacokinetic values**

| Total bilirubin (mg/dL) | $C_{max}$ (ng/mL)# | $AUC_{0-t}$ (hr*ng/mL)# | $AUC_{0-\infty}$ (hr*ng/mL)# | T½ (hr)# |
|---|---|---|---|---|
| ≤0.4 (n=6) | 14.220 (±4.498) | 269.827 (±160.335) | 286.286 (±161.657) | 30.537 (±9.211) |
| 0.41–0.7 (n=8) | 17.124 (±9.278) | 309.462 (±306.952) | 332.354 (±328.117) | 31.073 (±7.876) |
| 0.71–0.9 (n=4) | 19.460 (±3.391) | 421.798 (±161.345) | 443.610 (±206.016) | 35.073 (±16.790) |
| Above 0.9 (n=5) | 20.337 (±9.990) | 445.591 (±89.494) | 500.453 (±96.516) | 45.249 (±4.694) |

#Mean (±SD). AUC: Area under the curve, SD: Standard deviation

**Table 9: Summary of coefficient of correlation for clinical parameters with PK values**

| PK Parameters | Age (years) | Serum creatinine (mg/dL) | Total bilirubin (mg/dL) |
|---|---|---|---|
| Tmax (hr) | −0.29868 | −0.31062 | −0.32798 |
| $C_{max}$ (ng/mL) | 0.902399 | 0.906874 | 0.937149 |
| $AUC_{0-t}$ (hr*ng/mL) | 0.89306 | 0.889785 | 0.940271 |
| $AUC_{0-\infty}$ (hr*ng/mL) | 0.890481 | 0.890775 | 0.942407 |
| T½ (hr) | 0.942815 | 0.912834 | 0.901782 |

PK: Pharmacokinetics, AUC: Area under the curve, SD: Standard deviation

Hematology parameters such as Hb, TRBC, and HCT also displayed a positive correlation with systemic tacrolimus concentrations. This could be due to the extensive binding of tacrolimus to RBC (Hb), and as a result, the distribution of tacrolimus into whole blood is strongly affected by hematocrit [23]. A decrease in Hb count increases the free drug concentrations, which increases the CL of tacrolimus, resulting in decreased overall exposure to the drug [24]. Further in this study, an increase in systemic exposure ($C_{max}$ and AUC) and the corresponding decrease in tacrolimus CL (higher half-life) with elevated total bilirubin count was observed. This can be supported by the fact that an elevated total bilirubin decreases the biliary excretion of tacrolimus, resulting in a decrease in the overall CL of the drug from systemic circulation [24].

## CONCLUSION

The overall study shows that the PK of tacrolimus has a significant correlation with clinical investigations or screening parameters. Diversity in the values of these parameters, which decide the overall health of study participants and their correlation with PK values, could be a potential reason for the systemic variability of the drugs. Even with the small sample size and the values of clinical parameters within a healthy range, significant variability and noticeable correlations were observed. Higher systemic exposures were observed at upper values for clinical parameters such as age, serum creatinine, total bilirubin, and Hb and vice versa. This study with a larger and less controlled population (patients) can further magnify the role of clinical pathology together with demography on systemic drug exposure. These correlations will assist clinicians in developing effective drug therapies, particularly for NTI medications intended for long-term use and will also enable pharmaceutical researchers to optimally design clinical studies that yield better outcomes and higher success rates.

## ETHICS CONSIDERATIONS

The study protocol was approved by Jamia Hamdard Institutional Ethics Committee (JHIEC; registration number: ECR/48/Inst/DL/2013/RR-22), Hamdard Nagar, New Delhi 110 062 (INDIA). Written consent was obtained from all the study participants before the initiation of the research.

## ACKNOWLEDGMENT

All the authors wish to acknowledge the support and facilities received from Sun Pharmaceutical Industries Ltd. India.

## FUNDING

No financial support was received.

## AUTHOR'S CONTRIBUTION

Study design, methodology, data collection, analysis, interpretation, and manuscript drafting were performed by Mr. Sajad Khaliq Dar. Prof. Mohd Akhtar and Dr. Arshad H. Khuroo supervised the study design, methodology, and interpretation of results and reviewed the final manuscript. Dr. Sudershan Kumar reviewed the final manuscript.

## DECLARATION OF CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## REFERENCES

1. Haidar SH, Makhlouf F, Schuirmann DJ, Hyslop T, Davit B, Conner D, *et al*. Evaluation of a scaling approach for the bioequivalence of highly variable drugs. AAPS J. 2008 Sep;10(3):450-4. doi: 10.1208/s12248-008-9053-4, PMID 18726698
2. US Food and Drug Administration. Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products-General Considerations. US Food and Drug Administration; 2021.
3. FDA. Individual Product Bioequivalence Recommendations-Warfarin Sodium. Available from: https://fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm201283.pdf.2012
4. Health Canada. Comparative Bioavailability Standards: Formulations Used for Systemic Effects; 2018. Available from https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/dhp-mps/alt_formats/pdf/prodpharma/applic-demande/guide-ld/bio/comparative-bioavailability-standards-formulations-used-systemic-effects.pdf [Last accessed on 2025 Mar 25].
5. Japan Pharmaceutical, Food Safety Bureau. Guideline for Bioequivalence Studies for Different Strengths of Oral Solid Dosage forms. Available from: https://www.nihs.go.jp/drug/be-guide(e)/strength/gl-e_120229_ganryo.pdf.2012 [Last accessed on 2025 Mar 25].
6. Sam H. Narrow therapeutic index drugs: Clinical pharmacology perspective. J Pharm Pharmacol. 2021;73:1285-1291.
7. European Medicines Agency. Questions and answers: Positions on Specific Questions Addressed to the Pharmacokinetics Working Party. Available from: https://www.ema.europa.eu/docs/en_gb/document_library/scientific_guideline/2009/09/wc500002963.pdf.2013
8. Mohanan J, Palanichamy S, Kuttalingam A, Narayanasamy D. Development and characterization of tacrolimus tablet formulations for sublingual administration. Int J Appl Pharm. 2021;13(6):89-97. doi: 10.22159/ijap.2021v13i6.42429
9. Marshall DJ, Kim JJ, Brand S, Bryne C, Keevil BG. Assessment of tacrolimus and creatinine concentration collected using Mitra microsampling devices. Ann Clin Biochem. 2020;57(5):389-96. doi: 10.1177/0004563220948886, PMID 32713180
10. Van Gelder T, Gelinck A, Meziyerh S, De Vries AP, Moes DJ. Therapeutic drug monitoring of tacrolimus after kidney transplantation: Trough concentration or area under curve-based monitoring. Br J Clin Pharmacol. 2024;91:1600-6. doi: 10.1111/bcp.16098
11. Nacif LS, David AI, Pinheiro RS, Diniz MA, Andraus W, Cruz RJ, *et al*. An analysis of tacrolimus-related complications in the first 30 days after liver transplantation. Clinics (Sao Paulo). 2014;69(11):745-9. doi: 10.6061/clinics/2014(11)07. PMID 25518032
12. Thervet E, Loriot MA, Barbier S, Buchler M, Ficheux M, Choukroun G, *et al*. Optimization of initial tacrolimus dose using pharmacogenetic testing. Clin Pharmacol Ther. 2010;87(6):721-6. doi: 10.1038/clpt.2010.17, PMID 20393454
13. Andreu F, Colom H, Elens L, Van Gelder T, Van Schaik RH, Hesselink DA, *et al*. A new CYP3A5*3 and CYP3A4*22 cluster influencing tacrolimus target concentrations: A population approach. Clin Pharmacokinet. 2017;56(8):963-75. doi: 10.1007/s40262-016-0491-3, PMID 28050888
14. Andrews LM, Hesselink DA, Van Schaik RH, Van Gelder T, De Fijter JW, Lloberas N, *et al*. A population pharmacokinetic model to predict the individual starting dose of tacrolimus in adult renal transplant recipients. Br J Clin Pharmacol. 2019;85(3):601-15. doi: 10.1111/bcp.13838, PMID 30552703
15. Nuchjumroon A, Vadcharavivad S, Singhan W, Poosoonthornsri M, Chancharoenthana W, Udomkarnjananun S, *et al*. Comparison of tacrolimus intra-patient variability during 6-12 months after kidney transplantation between CYP3A5 expressers and nonexpressers. J Clin Med. 2022;11(21):6320. doi: 10.3390/jcm11216320, PMID 36362548
16. Mangoni AA, Jackson SH. Age-related changes in pharmacokinetics and pharmacodynamics: Basic principles and practical applications. Br J Clin Pharmacol. 2004;57(1):6-14. doi: 10.1046/j.1365-2125.2003.02007.x, PMID 14678335
17. Turnheim K. When drug therapy gets old: Pharmacokinetics and pharmacodynamics in the elderly. Exp Gerontol. 2003;38(8):843-53. doi: 10.1016/S0531-5565(03)00133-5, PMID 12915206
18. Corsonello A, Pedone C, Incalzi RA. Age-related pharmacokinetic and pharmacodynamic changes and related risk of adverse drug reactions. Curr Med Chem. 2010;17(6):571-84. doi: 10.2174/092986710790416326, PMID 20015034
19. Hudson JQ, Nolin TD. Pragmatic use of kidney function estimates for drug dosing: The tide is turning. Adv Chronic Kidney Dis. 2018;25(1):14-20. doi: 10.1053/j.ackd.2017.10.003, PMID 29499882
20. Munar MY, Singh H. Drug dosing adjustments in patients with chronic kidney disease. Am Fam Physician. 2007 May 15;75(10):1487-96.
21. Shah J, Fogel J, Balsam L. Importance of creatinine clearance for drug dosing in nursing home residents. Ren Fail. 2014;36(1):46-9. doi: 10.3109/0886022X.2013.831719, PMID 24111696
22. Vanaja K, Lavanya YR, Latha JM. A clinical comparative study of renal parameters of obese and hypertensive patients with non-obese hypertensive patients. Int J Curr Pharm Res. 2025;17(1):94-7. doi: 10.22159/ijcpr.2025v17i1.6057
23. Sikma MA, Van Maarseveen EM, Hunault CC, Moreno JM, Van de Graaf EA, Kirkels JH, *et al*. Unbound plasma, total plasma, and whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation. Clin Pharmacokinet. 2020;59(6):771-80. doi: 10.1007/s40262-019-00854-1, PMID 31840222
24. Tharanon V, Iamrahong P, Tuamsem J, Choochaeam K, Auamnoy T, Sobhonslidsuk A. Hemoglobin and total bilirubin may affect tacrolimus clearance in liver transplant patient following the early postoperative period: A case report. J Med Case Rep. 2024;18(1):408. doi: 10.1186/s13256-024-04753-3, PMID 39232822