PATTERNS OF TACROLIMUS METABOLISM AND KIDNEY ALLOGRAFT OUTCOMES AT THE KENYATTA NATIONAL HOSPITAL

Davies Otieno

MBChB (UoN), MSc (UK)

H58/37708/2020

A Dissertation Submitted in Partial Fulfilment for the Award of the Degree of the Master of Medicine in Internal Medicine, University of Nairobi

2023

STUDENT'S DECLARATION:

Principal Investigator:

Dr. Davies Otieno

Registrar, Internal Medicine

Department of Clinical Medicine and Therapeutics

Faculty of Health Sciences

University of Nairobi

Registration number: H58/37708/2020

I, Davies Otieno, do declare that this is my original work and has not been submitted for academic or career pursuits in any other institution.

Signed: ..... **Date:** 6th November 2023

SUPERVISORS' APPROVAL:

This dissertation has been submitted with our approval as the supervisors and the chairman from the Department of Clinical Medicine and Therapeutics, Faculty of Health Sciences, University of Nairobi

SUPERVISORS:

Prof. Seth O. McLigeyo

Professor

Consultant Physician and Nephrologist,

Department of Clinical Medicine, and Therapeutics, University of Nairobi

Signed: ..... **Date:** 6/11/23

Prof. Joshua K. Kayima

Associate Professor

Consultant Physician and Nephrologist

Department of Clinical Medicine, and Therapeutics, University of Nairobi

Signed: ..... **Date:** 06/12/2023

Dr. Enoch O. Omonge

Senior Lecturer

Consultant Physician and HIV specialist

Department of Clinical Medicine and Therapeutics, Unit of Clinical Pharmacology

University of Nairobi

Signed: ..... **Date:** 6/11/23

Dr. Syokau Ilovi

Lecturer,

Consultant Physician and Medical Geneticist,

Department of Clinical Medicine, and Therapeutics,

University of Nairobi

Signed: ..... **Date:** 7.11.2023

CHAIRMAN OF THE DEPARTMENT:

Prof. E. O. Amayo

Chairman

Consultant Physician and Neurologist

Department of Clinical Medicine, and Therapeutics,

University of Nairobi

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF CLINICAL MEDICINE & THERAPEUTICS
P.O. BOX 19676-00202 NAIROBI

Signed: ..... **Date:** 9/11/2023

ACKNOWLEDGEMENT:

To the Almighty God to whom I am eternally beholden, thank you.

I would like to extend my gratitude to my family, friends, the faculty members at the Department of Clinical Medicine and Therapeutics and consultants in the Renal Unit, Kenyatta National Hospital for encouragement and advice during the study period. Sincere appreciation to the Kenyatta National Hospital, Medical Research Department for funding this study.

DEDICATION:

I would like to dedicate this dissertation to my parents (Frederick C.F. Otieno and Beatrice Osino) and brother (Alfred Omondi).

Table of Contents:

LIST OF ABBREVIATIONS:

ABCB1	adenosine triphosphate-binding cassette subfamily B member 1
ACMIA	antibody conjugated magnetic immunoassay
ADME	absorption distribution metabolism excretion
AUC	area under the curve
BMI	body mass index
BPR	biopsy proven rejection
C_0	tacrolimus trough concentration
CKD	chronic kidney disease
CLIA	chemiluminescent enzyme immunoassay
CMIA	chemiluminescent microparticle immunoassay
CMV	Cytomegalovirus
CNIs	calcineurin inhibitors
CNS	central nervous system
C_0/D ratio	tacrolimus concentration-dose ratio / dose-adjusted tacrolimus trough concentration
CYP3A5	cytochrome P450 3A5
D	tacrolimus dose per day in milligrams
DAF	delayed allograft function
DMET	drug metabolising enzymes and transporters
DTPA	diethylenetriamine pentaacetate
ECLIA	electrochemiluminescence immunoassay
EDTA	ethylenediaminetetraacetic acid
eGFR	estimated glomerular filtration rate
EMIT	enzyme-multiplied immunoassay technique
ELISA	enzyme linked immunosorbent assay
ERC	Ethical Review Committee
ESKD	end stage kidney disease
FKBP	FK506 Binding Protein
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSCT	hematopoietic stem cell transplantation

IM	Intermediate tacrolimus metaboliser
KAR	kidney allograft recipients
KNH	Kenyatta National Hospital
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LMIC	low middle income countries
MDRD	modification of diet in renal disease
MDR1	multidrug resistance protein 1
MHC	major histocompatibility complex
MPA	mycophenolic acid analogues
mTOR	mammalian Target of Rapamycin
PPI	proton pump inhibitor
PTDM	post-transplant diabetes mellitus
PXR	pregnane X receptor
RBC	red blood cell
RTM	rapid tacrolimus metaboliser
STM	slow tacrolimus metaboliser
SOT	solid organ transplantation
SPSS	statistical packages for the social sciences
SRTR	Scientific Registry of Transplant Recipients
TB	tuberculosis
TDM	therapeutic drug monitoring
UoN	University of Nairobi

OPERATIONAL DEFINITIONS:

Dose-adjusted tacrolimus trough concentration (C_0/D) ratio – An index derived by dividing the tacrolimus trough concentration by the total tacrolimus daily dose (mg). This is used to classify patients into 3 groups according to their capacity to metabolise tacrolimus.

Delayed allograft function - Reversion to dialysis within the first week of transplantation.

Dialysis vintage – Duration of dialysis (in months) prior to transplantation as documented in the file.

Incomplete medical charts – Less than five recordings of creatinine levels, tacrolimus trough levels and corresponding daily doses recorded in the files during the first year of kidney transplantation (at hospital discharge, at one month, three months, six months and at one year)

Patterns of tacrolimus metabolism - A classification system derived from C_0/D ratio cut-off values used to assign patients their metabolic phenotype (slow, intermediate, and rapid) and corresponding CYP3A5 genotype.

Tacrolimus metabolising status / metabolic phenotype – Strata within the classification system ascribed after applying the C_0/D ratio cut-off values (slow, intermediate, and rapid metabolisers).

LIST OF FIGURES:

LIST OF TABLES:

LIST OF EQUATIONS:

ABSTRACT:

Background:

Tacrolimus is a key immunosuppressive agent used in kidney transplantation. It is characterised by marked between- and within-patient variabilities due to genetic and non-genetic factors. The main drug metabolising enzyme of tacrolimus, CYP3A5, is highly polymorphic resulting in varying metabolic phenotypes in the population. The dose-adjusted tacrolimus trough concentration (C_0/D ratio) allows for prediction of the metabolic phenotype among kidney allograft recipients on therapeutic drug monitoring, without genotypic testing. The study set out to determine the percentage of rapid, intermediate, and slow metabolisers of tacrolimus by calculating C_0/D ratio among kidney allograft recipients at the Kenyatta National Hospital in Nairobi, Kenya.

Materials and methods:

A retrospective chart review of kidney allograft recipients' files taking tacrolimus for maintenance transplant immunosuppression at the KNH was conducted. The recipients' age, sex, pre-transplant comorbidities and use of induction therapy were recorded. Tacrolimus total daily dose (D), trough concentrations (C_0) and allograft function were recorded at discharge, within one month, three months, six months and at one year of kidney transplantation. The C_0/D ratio was calculated using tacrolimus trough and total daily dose at indicated time points and an average value obtained for each patient. Allograft function was deduced from the Modification of Diet in Renal Disease (MDRD-4) formula.

Results:

Among the 95 kidney allograft recipients' medical records reviewed, 67 (70.5%) were males. The mean age was 35±13.3 years, and a median dialysis vintage time of 19 months (range 0 to 75). About 51 (53.7%) and 42 (44.2%) had pre-morbid hypertension and glomerulonephritis respectively, while 12 (12.6%) had diabetes mellitus. With the interpretation of C_0/D ratio as <1.05 as rapid metabolisers, 1.05-1.54 as intermediate and >1.55 as slow metabolisers, there were 40 (42.1%) rapid metabolisers, 23 (24.2%) intermediate and 32 (33.7%) slow metabolisers (p-value <0.001). Rapid metabolisers had the lowest mean tacrolimus trough concentration (7.0 ± 1.5 ng/ml, p value< 0.001) and the highest tacrolimus dose requirement (8.8 ± 2.0 mg/day), p value < 0.001).

Conclusion and recommendation:

The cohort consisted of majorly of rapid metabolisers followed by slow metabolisers, based on C_0/D ratio. These metabolic phenotypes may be interpreted to mean that majority of the recipients will require tacrolimus dosing using the higher limit of the dosage range. More studies on tacrolimus metabolism are required in the transplant population in this setting.

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Kidney transplantation is the preferred treatment modality for patients with end stage kidney disease (ESKD), one resulting in long term cost-effectiveness and overall better quality of life. Its success primarily depends on optimal immunosuppression to minimise allograft rejection while guarding against cardiovascular, infectious, and neoplastic consequences.

Transplant immunosuppression occurs in three phases: induction, maintenance, and treatment for acute rejection. Broadly, induction therapy involves depletional and non-depletional agents such as antithymocyte globulin (ATG) and basiliximab respectively. Maintenance therapy comprises the use of corticosteroids such as prednisone and methylprednisolone, mammalian target of rapamycin (mTOR) inhibitors such as everolimus and sirolimus, antiproliferative agents such as azathioprine and mycophenolate mofetil and calcineurin inhibitors (CNIs) such as cyclosporine-A and tacrolimus. These agents target antibody- and T-cell mediated responses, key effectors of the immune response to rejection. Combination therapy, using three drugs from different drug classes, popularly referred to as triple immunosuppression, has been the standard of care for transplant-associated immune suppression therapy, with transplant centres employing different drug combinations to prevent allograft rejection and loss(1,2).

Calcineurin inhibitors have formed the backbone of immunosuppression treatment protocols worldwide, with tacrolimus-based combinations favoured over cyclosporine-A -based regimens due to reduced episodes of rejection, cost-effectiveness, and favourable toxicity profile(3). However, no differences in the rates of allograft survival between the calcineurin inhibitors has been demonstrated(4). Locally, the most common calcineurin inhibitor prescribed for use by kidney allograft recipient(s) is tacrolimus.

Tacrolimus is known to have a narrow therapeutic range and marked inter- and intra-individual variabilities. These characteristics necessitate the need for tacrolimus therapeutic drug monitoring (TDM) in kidney allograft recipients, especially due to concurrent medications and attendant food-drug and drug-drug interactions. Tacrolimus area under the curve (AUC) is regarded as the parameter that best describes tacrolimus' exposure(5), however, pre-dose or trough tacrolimus concentration in blood is preferred for TDM due to its ability to correlate with AUC, its fair cost and feasibility in clinical practice.

Tacrolimus trough concentrations are the result of several factors, especially by determinants of drug metabolism. These factors play a role in the variability of tacrolimus trough levels observed in clinical practice. Pharmacogenetics of drug metabolising enzymes and transporters (DMETs), especially CYP3A5 gene family, is associated with close to 40% of the variability with clinical correlates accounting for 46%(6). A recent study to determine tacrolimus and cyclosporine A trough levels in blood samples of patients taking these medications at a major laboratory in Kenya demonstrated that a total of 32% of samples had sub-optimal and toxic drug levels(7), possibly due to polymorphisms expressed by rapid and slow metabolisers. This also emphasizes the need for therapeutic drug monitoring and rational dose adjustment. Patients with suboptimal serum tacrolimus levels risk developing allograft rejection while those with toxic drug levels risk adverse effects such as allograft nephropathy, all culminating in allograft loss and need for return to dialysis and/or re-transplantation. It is therefore important to maintain tacrolimus levels within the therapeutic range, especially in the first year of transplantation, to reduce the risk of acute rejection, allograft injury and infectious complications(8). Understanding the sources of variability in tacrolimus disposition will enable transplant physicians develop and implement actionable strategies to optimise allograft outcomes.

In view of the influence of genetic factors, metabolic phenotypes correlating with CYP3A5 pharmacogene expression have been previously described and a prognostic value impacting on kidney allograft and patient survival attached to these phenotypes(9–11).

1.2 Patterns of tacrolimus metabolism

Individuals are classified as slow, intermediate, and rapid metabolisers of tacrolimus based on a clinical index, the dose-adjusted tacrolimus trough concentration (C_0/D ratio). In centres where genotypic testing is not readily available and would be expensive to procure, this index has the potential to predict the metabolic genotypes and phenotypes with the intention of informing rational dose adjustment and therefore optimise allograft survival. The table below summarises the metabolic phenotype and the corresponding CYP3A5 genetic polymorphisms according to the Clinical Pharmacogenetics Implementation Consortium (CPIC)(9).

Table 1. Metabolic phenotypes, C_0/D ratio cut-off values and corresponding CYP3A5 diplotype

Phenotype (C_0/D ratio)	Corresponding diplotype
Rapid tacrolimus metabolisers (<1.05)	CYP3A5*1/*1
Intermediate tacrolimus metabolisers (1.05 to 1.54)	CYP3A5*1/*3, CYP3A5*1/*6, CYP3A5*1/*7
Slow tacrolimus metabolisers (>1.55)	CYP3A5*3/*3, *6/*6, *7/*7, *3/*6, *3/*7, *6/*7

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Immunosuppressive regimens

Ideal immunosuppression with regards to transplant clinical practice refers to pharmacological inhibition that achieves allograft acceptance with minimal interference of systemic immunity to other foreign molecules(12). Combination therapy is prescribed to minimise the harmful effects associated with individual drugs while maximising on synergistic effects. Regimens vary worldwide according to various factors such as organ transplant type, side effect profile and co-morbid conditions. Kidney transplant protocols favour CNI-based regimens, particularly tacrolimus over cyclosporine(13).

2.2 Calcineurin inhibitors (CNIs)

Calcineurin is a calcium-calmodulin dependent serine/threonine phosphatase that dephosphorylates nuclear factor of activated T-cell (NFAT) upon T-cell receptor (TCR) stimulation. Peptide binding to TCR/CD3 complex during antigen presentation by major histocompatibility complex (MHC) molecules stimulates nuclear factor of activated T-cell (NFAT) translocation to the nucleus to induce T-cell activation through regulation of genes responsible for cytokine synthesis as depicted in figure 1. Calcineurin inhibitors bind to cytosolic peptidyl prolyl isomerases, also known as immunophilins, and form a complex that inhibits calcineurin resulting in attenuation of T-cell activity thus preventing allostimulation and subsequent immune activation(13).

Although they have similar mechanisms of action of binding to immunophilins, the class members bind to different immunophilins, whose classification into two families is based on their pharmacological target(14): FK506-binding proteins (FKBPs) for pimecrolimus and tacrolimus and cyclosporin-binding cyclophilins (CyPs) for cyclosporine. Amongst the three, tacrolimus is the preferred calcineurin inhibitor for use in solid organ transplantation, including kidney transplantation.

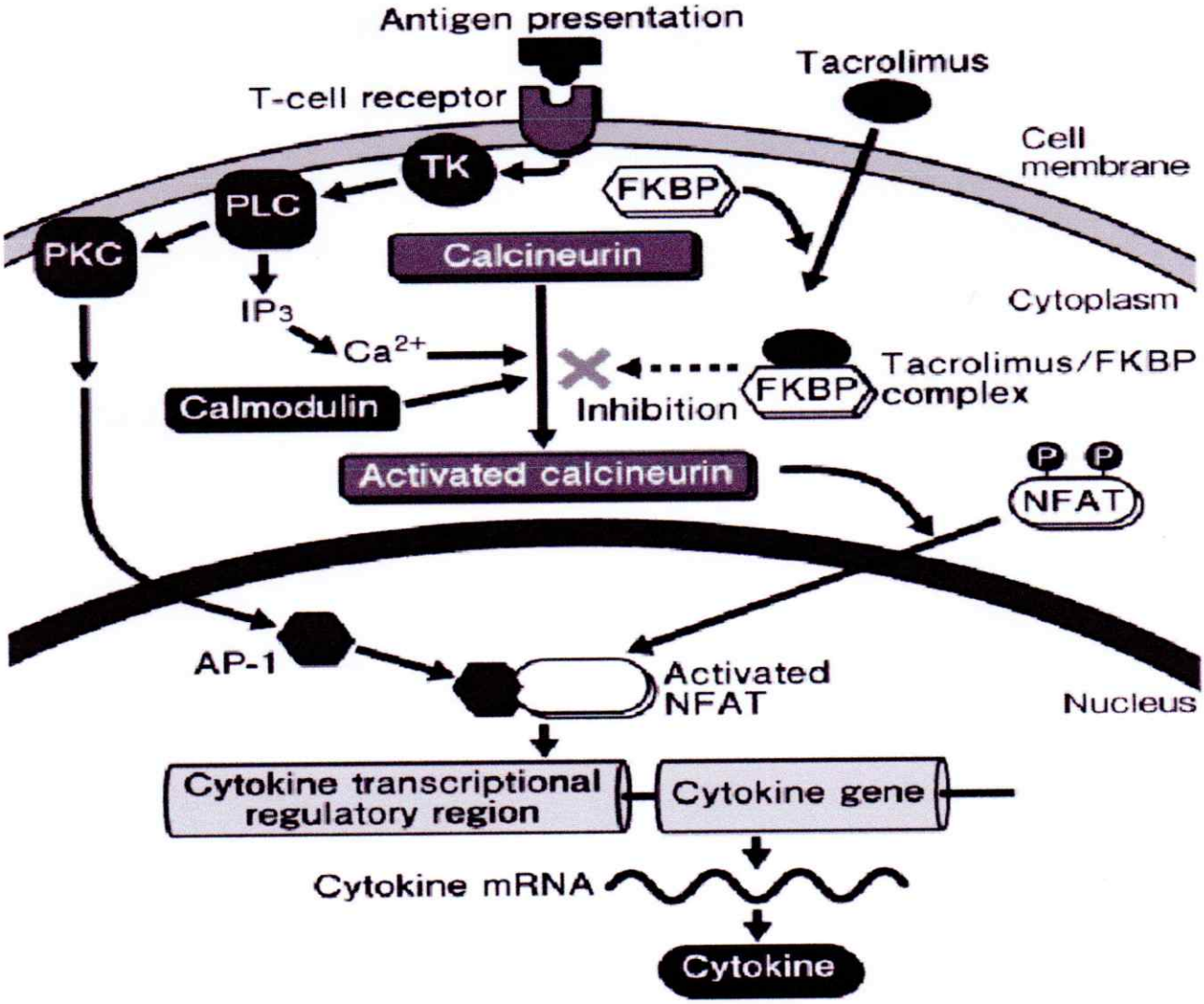


Figure 1. Mechanism of action of tacrolimus.

(Available from: https://www.researchgate.net/figure/Mechanism-of-action-for-tacrolimus-FKBP-FK506binding-protein-NFAT-nuclear-factor-of_fig1_325914850)

2.3 Tacrolimus

In 1984, a 23-membered macrolide lactone antibiotic was derived by researchers at Chiba University of Japan from Streptomyces tsukubaensis, a soil fungus found in the foot of Mt. Tsukuba in Tokyo, Japan. It was initially approved for use by the United States Food and Drug Administration (US-FDA) in 1994 for the prevention of rejection in liver transplant recipients. Ensuing clinical trials in kidney transplantation demonstrated similar benefit and led to its approval for that use in the year 1997. Subsequent clinical trials conducted shortly after led to its use in cardiac transplantation, and until recently, in July 2021, it received FDA approval for use in lung transplant recipients after secondary analysis of the Scientific Registry of Transplant Recipients (SRTR) provided real-world-evidence regarding its efficacy(15). A review of its pharmacokinetics will elucidate potential levels for explaining its variability.

2.3.1 Pharmacokinetics

2.3.1.1 Absorption

Various formulations of tacrolimus exist for intravenous, oral, sublingual, and topical administration. Although intravenous and sublingual tacrolimus formulations present alternative options for its administration(16,17), the oral route is preferred for use in kidney transplantation. The formulations come in once-daily prolonged-release gelatin capsules, for instance, Advagraf and twice-daily immediate-release capsules such as Prograf. The most prescribed formulation in Kenya is yet to be determined as data is not available.

The oral bioavailability of tacrolimus varies from 4% to 89% depending on the formulation used, concomitant food-drug and drug-drug interactions(18). This is partly attributed to its poor solubility and high lipophilicity, in addition to extensive hepatic and intestinal first-pass effect by cytochrome P450 (CYP) enzyme subfamily 3A5 and the efflux transporter, P-glycoprotein (ABCB1/MDR1), encoded by ABCB1 gene. Additionally, in healthy volunteers, a statistically significant difference in relative bioavailability was noted, especially between the fasted state and a post-prandial state after a fat-rich diet(19).

Maximum CYP3A enzyme expression occurs in the liver. Conversely, it decreases in concentration distally in the gastro-intestinal tract while P-glycoprotein expression demonstrates an antegrade increase(20). Food increases time to maximum concentration by altering gastric pH, hepatic and

splanchnic circulation, and forms complexes that act as barriers to absorption(21). Altered gastric pH due to co-administration with proton pump inhibitors (PPIs) reduces tacrolimus exposure in kidney allograft recipients, especially in those with CYP2C19 gene mutations(22). However, a similar effect was not observed in liver transplant recipients although there seems to be intra-class differences(23,24).

Hyperperistalsis due to non-infectious and infectious diarrhoea decreases intestinal passage time. This increases tacrolimus delivery to the distal ileum and colon, where maximum absorption occurs by passive diffusion as demonstrated by stable isotope labelling in healthy volunteers(25), thus elevating tacrolimus AUC, peak and trough concentrations(26,27). Conversely, inflammation downregulates the activity of P-glycoprotein and CYP enzyme family thus increasing CNI levels(28). Gut microbiota has also been shown to affect tacrolimus absorption by increasing its dosing requirements through various mechanisms such catabolism by faecal Faecalibacterium prausnitzii into less potent metabolites(29).

2.3.1.2 Distribution

Tacrolimus exhibits a high blood to plasma ratio because of its lipophilicity and high affinity for erythrocyte immunophilins. It is extensively bound to red blood cells therefore demonstrating a non-linear relationship at low haematocrit and high drug concentrations due to saturable mechanisms. This predisposes patients to drug toxicity(30). For this reason, tacrolimus has a low apparent volume of distribution and is assayed in whole blood for therapeutic drug monitoring. The remaining fraction of tacrolimus is found in plasma, 99% of which is bound to predominantly albumin, α-1-glycoprotein, and lipoproteins, leaving less than 1% of active or unbound tacrolimus(31). Subsequent equilibration of unbound tacrolimus in body compartments occurs.

Site-specific tissue concentration is determined by the distribution of P-glycoprotein and immunophilins. For instance, single-nucleotide polymorphisms on the ABCB1 gene were shown to reduce the efflux of intra-lymphocytic tacrolimus, thereby enhancing the immunosuppressive effect and prevention of allograft rejection(32). Similarly, P-glycoprotein non-expression at the blood-brain barrier was demonstrated to increase the uptake of tacrolimus into the central nervous system (CNS) in MDR1 gene knockout mice, a possible mechanism mediating neurotoxic effects of CNIs(33).

2.3.1.3 Metabolism and elimination

The primary sites of tacrolimus biotransformation are the liver and intestine due to abundance of cytochrome P450 enzyme family, CYP3A5. The drug undergoes extensive demethylation and

hydroxylation into approximately 13 metabolites, the main one being 13-O-demethyl tacrolimus (M-I) with approximately 15 times less potent immunosuppressive effects than the parent compound(34). Other common metabolites include 31-O-demethyl-tacrolimus (M-II) and 15-O-demethyl-tacrolimus (M-III). However, these metabolites have been speculated to mediate nephrotoxicity and myelosuppression(35).

A dose-dependent sex disparity in tacrolimus metabolism has been reported, with females demonstrating lower AUC levels than men, although the difference may be explained by additional factors such as body weight and drug-drug interactions(36,37). Conversely, steroid-minimisation has been shown to increase tacrolimus concentration and therefore exposure, due to withdrawal of steroid effect on pregnane X receptor (PXR)(38). Similarly, mycophenolic acid analogues (MPA) have been shown to increase tacrolimus exposure however the mechanism is yet to be elucidated(39).

Approximately 0.5% of unmetabolized tacrolimus is eliminated through the stool and urine. Intra-renal metabolism of tacrolimus has been shown to occur due to the preferential expression of CYP3A5 over CYP3A4 on tubular cells(40). A plausible clinical implication of this localisation is that CYP3A5 expressors are predisposed to CNI nephrotoxicity although conflicting results in other solid organ transplants have been reported(41,42).

2.4 Tacrolimus assay techniques

Monitoring of tacrolimus levels is part of standard of care in solid organ transplantation (SOT) protocols worldwide. Due to the implication of the ensuing result, assay techniques need to be validated and personnel adequately trained to handle the reagents and equipment used. Two methods of quantification exist, mass spectrometry and immunoassays. However, due to lack of harmonization of bench procedures, non-adherence to consensus guidelines and poor uptake of reference reagents, there also exists inter-laboratory variability(43).

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) with electrospray ionization is the preferred modality due to its sensitivity for the parent compound and ability to analyse tacrolimus metabolites without cross-reactivity(5). Additionally, this method can perform multianalyte analysis in a single sample. However, it is expensive to procure and requires highly skilled personnel to operate and interpret the results. Immunoassay methods present a cheaper alternative where monoclonal antibodies are used to recognise different epitopes of the tacrolimus molecule. These techniques are

harmonised through manufacturer's calibration, can be automated, and provide a rapid TAT (turn-around-time).

Commonly used immunoassays include antibody conjugated magnetic immunoassay (ACMIA), chemiluminescent enzyme immunoassay (CLIA), electrochemiluminescence immunoassay (ECLIA) and enzyme-multiplied immunoassay technique (EMIT). Due to noteworthy cross-reactivity with tacrolimus metabolites (M-I, M-II and MIII) resulting in falsely elevated tacrolimus levels, the use of EMIT for TDM has reduced(44). Hypoalbuminemia has been demonstrated to overestimate tacrolimus levels in the early post-operative period when quantified using ACMIA, risking improper dose modification(45). ECLIA has been shown to perform with a similar sensitivity to LC-MS/MS by its ability to detect tacrolimus concentrations at the lower limit of quantification(46). At the Kenyatta National Hospital, immunoassay methods have predominated since the initiation of the kidney transplant program, with CLIA having been in use for about 10 years and chemiluminescent microparticle immunoassay (CMIA) being the current method for tacrolimus quantification in blood.

2.5 Determinants of tacrolimus trough concentration

Marked between- and within-patient variability in tacrolimus trough levels is a recognised phenomenon and is attributed to multiple factors: genetic, demographic, food-drug, drug-drug and disease-drug interactions and the type of assay employed to quantify the drug. Clinically significant variability observed with tacrolimus has been described as the in- and out-of-range tacrolimus levels observed over a period where the dose remains unchanged. Veritably, high tacrolimus variability, more so, intra-patient variability, correlates with development of donor specific antibodies in kidney transplantation resulting in unfavourable allograft outcomes(47). Genetic factors account for 39% of the variability while non-genetic factors (clinical and demographic correlates) explain the majority, at 46%(6).

It is important to investigate the role of genetic factors in our setup to gain insight into local tacrolimus disposition characteristics. However, several barriers to routine pharmacogenetic testing exist, such as equipment cost, lack of expertise and the lack of consensus guidelines for interpretation of results, especially with significant ethnic variation. A previously described index, the dose-adjusted tacrolimus trough concentration (C_0/D), has been shown to correlate with CYP3A5 expression and allograft outcome in kidney transplantation at steady state(9,48). This tool has the potential to account for local tacrolimus disposition in our locale, and its relationship with infectious, cardiovascular and allograft outcomes.

2.6 Determinants of kidney allograft function

In addition to the role played by pharmacological suppression, allograft function as measured by serum creatinine has been shown to be impacted by other diverse factors, classified as pre-transplant donor and recipient factors, peri-operative and post-transplantation factors. Pre-morbid conditions, donor-type, age and gender of donor and recipient, dialysis vintage, number of human leukocyte antigen (HLA) mismatches, prior sensitization from blood transfusion and pregnancy and medication non-adherence are some of the recognised factors demonstrated to influence allograft survival. Although the individual contribution of each factor appears negligible, their summation impact allograft survival significantly(49). The figure below summarises the known factors and the time-point within which they are significant in determining allograft survival.

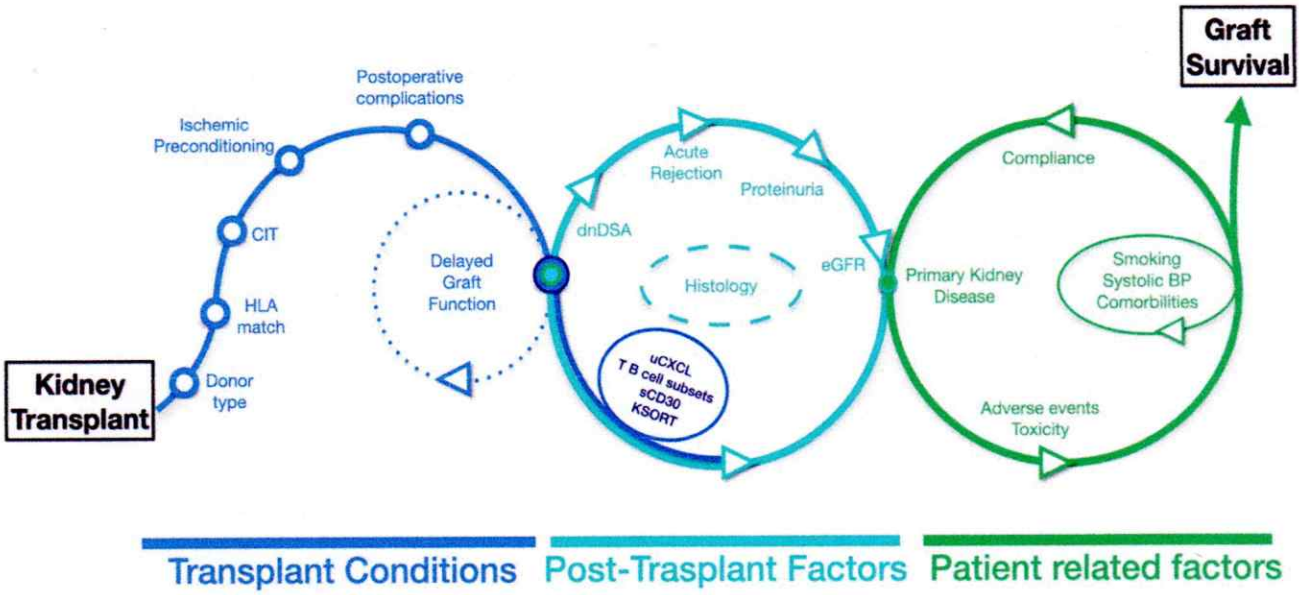


Figure 2. Cycle chart demonstrating some recognised predictors of graft function. Adopted from(50)

2.7 Study justification and significance

Kidney allograft survival is influenced by multiple factors in our clinical practice setup. Tacrolimus is key to immunosuppression, yet its use is associated with unique challenges. Populations exhibit genetic variations in drug disposition with high serum levels predisposing to drug toxicity while lower levels are associated with allograft rejection.

Local studies have explored the significance of non-genetic factors in determining allograft outcomes, none has yet investigated genetic determinants. Genotype testing to determine a patient's tacrolimus disposition characteristics is not a standard of care, however, there are surrogate markers used to predict this variation. A local study hypothesized that our population consists of slow metabolisers after examining tacrolimus dosing requirements and resultant trough levels within the first week of transplantation(51). The clinical index (C_0/D ratio) with genotype-predictive and prognostic value has not been previously employed in our local setup.

On that account, this study sought to provide novel insights into the genetic factors determining tacrolimus disposition in our setup. The study intends to influence the development of national guidelines in the management of kidney allograft recipients (KARs) that optimise allograft survival through rational prescribing and dose adjustments. This study will also emphasize the role of laboratory services in the management of kidney allograft recipients.

2.8 Research question

What is the effect of the pattern of tacrolimus metabolism on kidney allograft outcomes?

2.9 Study objectives

2.9.1 Broad objective

To establish the patterns of tacrolimus metabolism and relationship with allograft function among kidney allograft recipients at KNH within the first year of transplantation

2.9.2 Specific objectives

I. To determine the proportion of rapid, intermediate, and slow metabolisers of tacrolimus among kidney allograft recipients at the Kenyatta National Hospital

II. To determine the trends of allograft function using eGFR at selected time-points within the first year of transplantation in rapid, intermediate, and slow metabolisers of tacrolimus among kidney allograft recipients at the Kenyatta National Hospital

CHAPTER THREE

3. METHODOLOGY

3.1 Study design

A retrospective chart review

3.2 Study area

The study was conducted at the transplant wing of the KNH renal unit. KNH is the largest public teaching and referral hospital in Kenya. Founded in early 1900s with an initial bed capacity of 40, the hospital currently has a bed capacity of about 2000 and serves as the main teaching hospital for the University of Nairobi, Faculty of Health Sciences. It is the first public hospital in the country to run a kidney transplant unit, in partnership with Novartis through the Interlife program. The unit offers a weekly kidney transplant clinic, so far serving over 150 patients transplanted since the inception of the program.

3.3 Study population

The medical records of kidney allograft recipients (KARs) attending the KNH renal unit transplant clinic, having undergone living-donor kidney transplant surgery at the KNH under the Novartis Interlife Program, and are on taking tacrolimus as one of the immunosuppressive agents.

Three days preceding the scheduled transplant surgery, kidney allograft candidates begin taking tacrolimus and a mycophenolic acid analogue. The tacrolimus dose is initiated at 0.15mg/kg/day and titrated based on tacrolimus trough concentrations. The kidney allograft recipient is induced using intravenous methylprednisolone at a dose of 500mg at the release of clamps intraoperatively, 250mg on day one and 100mg on day two respectively, after which oral prednisone is started at a dose of 30mg per day as a single dose from day 3 and tapered off by 2.5mg daily until day 7 when the dose is 20mg. Basiliximab is administered on day-zero pre-operatively and day-four post-operatively to selected individuals whose HLA matches were less than three out of the six loci of HLA-A, -B and -DRB1.

Patients are discharged from hospital after one to two weeks on tacrolimus, mycophenolic acid, and prednisone as their immunosuppressive regimen. They receive cotrimoxazole and isoniazid prophylaxis for opportunistic infections. Medications for premorbid conditions are also continued. A steroid-tapering regimen is adhered to through the first year, with a goal of steroid minimisation.

Tacrolimus trough concentration, whose therapeutic reference range lies between 5ng/ml and 15ng/ml, is measured in whole blood from an ethylenediaminetetraacetic acid (EDTA) vacutainer one hour prior to the next tacrolimus dose. The method of assay used at the KNH Renal laboratory for drug monitoring is the competitive immunoluminometric technique, a type of CLIA, whose range of concentration detection is between <2ng/ml and 50ng/ml. Standard Operating Procedures, as stipulated by the manufacturer, are adhered to by qualified laboratory professionals when quantifying tacrolimus levels.

3.3.1 Selection criteria

Medical charts of KARs at the KNH:

- on a tacrolimus-based regimen for more than a year
- Sufficient records defined as having been followed up for a year or more with at least 2 clinic visits within the first 3 months of transplant surgery, one clinic visit after every three months for up to one year with documented tacrolimus trough concentration and dose(s) for each clinic visit.

3.3.2 Exclusion criteria

Medical records of KARs at the KNH:

- on a cyclosporine-based regimen
- who received a kidney allograft at a site different from the Kenyatta National Hospital

3.4 Sample size calculation

Cochrane's sample size formula for categorical data (Equation 1) was used to obtain the desired sample size (n_0), whereby,

Equation 1. Cochrane's sample size formula for categorical data

$$n_0 = \frac{(t)^2 * (p)(q)}{(d)^2}$$

t – value for selected alpha level (1.96)

p – proportion of rapid metabolisers (23%) (obtained from a population study of African Americans(52))

q – (1-p)

d - acceptable error margin – 0.05

- n_0= 272

272 exceeds 5% of the population, therefore a re-adjustment of sample size is undertaken using Cochrane's correction formula.

Equation 2. Cochrane's correction formula

$$n_1 = \frac{n_0}{(1 + n_0 / \text{Population})}$$

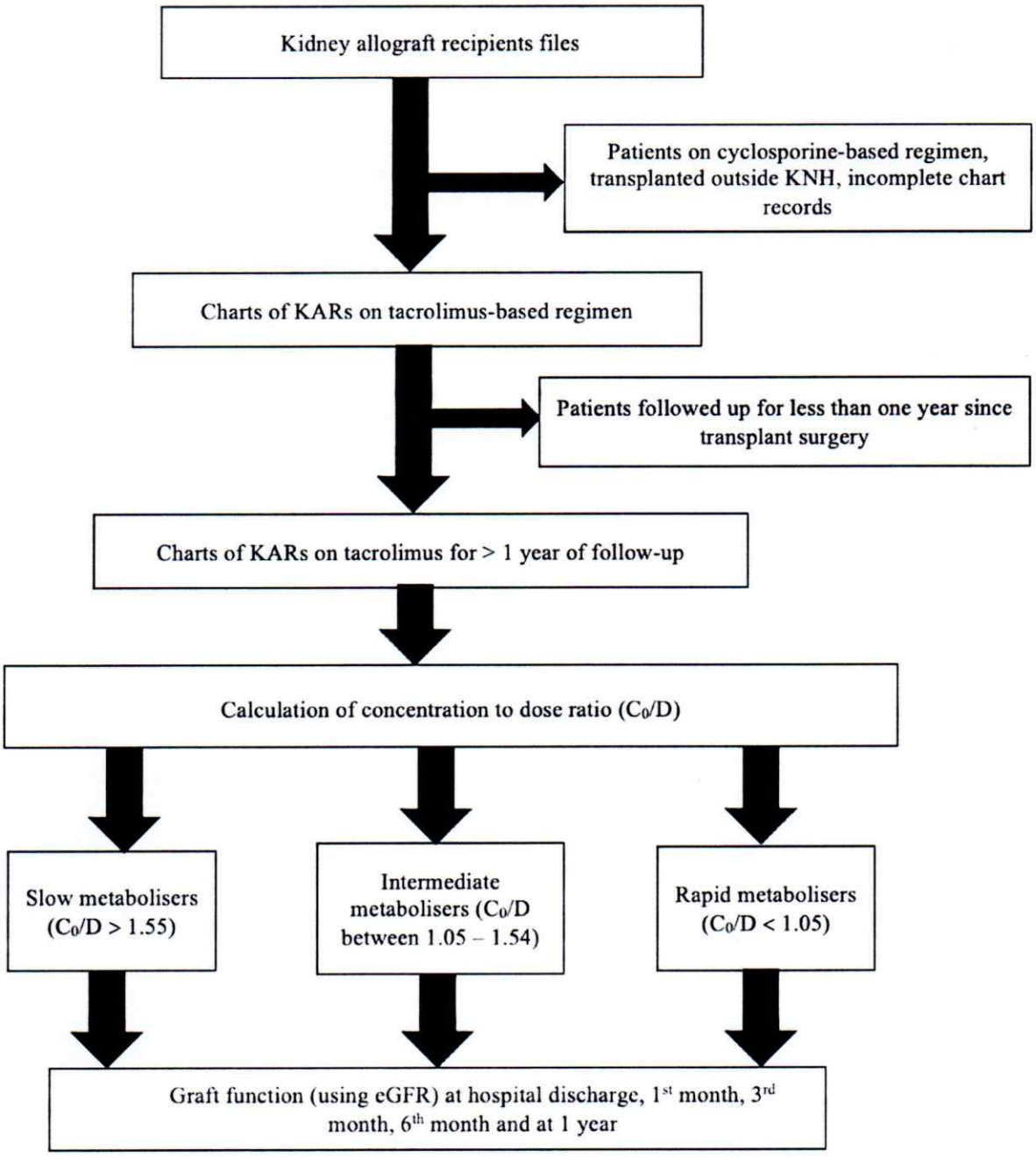
n_0 = 272

population of tacrolimus users = 110

n_1 = 78

Accounting for 10% information loss, a minimum sample size of 86 patients' charts was targeted. However, all patient charts were scrutinized for eligibility into the study.

3.5 Study flow chart diagram



Flowchart diagram (1) demonstrating the recruitment procedure.

3.6 Data Management

3.6.1 Data Collection Procedure

All KAR charts that met the inclusion criteria were reviewed. The principal investigator and a research assistant assessed the files and the following characteristics recorded in the data abstraction form (Appendix 1):

- Age of recipient at transplantation in completed years, date of living-donor kidney transplant surgery (date/month/year), sex of recipient, weight and height of the recipient, use of induction therapy, comorbid conditions, dialysis vintage and presence of delayed allograft function (DGF)
- The following parameters were recorded from every stipulated visit within the 1st month and at the 3rd, 6th, and 12th month visits: creatinine, tacrolimus trough concentration and dose, and steroid dosage.

Tacrolimus trough concentration and dose were recorded at hospital discharge from transplant surgery and during the following clinic visits: within the 1st month of transplant surgery and at 3rd, 6th, and 12th month. An allowance of 3 weeks was granted for the visits on the 3rd month and 1 month for the visits on the 6th and 12th month. In the presence of more than one value of the parameters of interest around the time of clinic visits, the average of the parameter was calculated and recorded. Tacrolimus trough concentrations recorded as the lowest limit of quantification of less than 2ng/mL were recorded as 1ng/ml. The dose-adjusted tacrolimus trough concentration (C_0/D ratio) was calculated using the formula below by Thölking, G. et al (53) as shown in equation 3 at hospital discharge, 1st ,3rd ,6th and 12th month and an single average obtained for each patient. The following cut-off values were used to stratify the participants based on their metabolising status: rapid metabolisers (C_0/D ratio < 1.05), slow metabolisers (C_0/D ratio > 1.55), and intermediate metabolisers (C_0/D ratio between 1.05 – 1.54).

Equation 3. Formula used to calculate tacrolimus dose-adjusted trough concentration (C_0/D ratio)

$$C/D\,ratio\,(ng/mL * 1/mg) = \frac{blood\ Tac\ trough\ level\,(ng/mL)}{daily\ Tac\ dose\,(mg)}$$

The study outcomes were stipulated as follows:

- Allograft function at the first, third and sixth month and at one year since transplant surgery was presented as calculated eGFR by the Modification of Diet in Renal Disease-4 (MDRD-4) formula using serum creatinine values at the respective time points.
- Calculated eGFR of > 60mls/min/1.73m^2 was regarded as good allograft function while a calculated eGFR of < 60mls/min/1/73m^2 was regarded as poor allograft function.

3.6.2 Study Variables

Demographic variables: Age of recipient at transplantation, sex of recipient, recipient weight, height, and calculated body mass index (BMI)(kg/m^2), use of induction therapy, dialysis vintage in months

Medication related variables: Tacrolimus average trough concentration and dosage, steroid use and dosage, metabolic phenotype classified as slow, intermediate, and rapid, using C_0/D ratio cut off values.

Outcome variable: eGFR – estimated glomerular filtration rate as calculated by the MDRD-4 formula.

3.6.3 Data Analysis

Data was extracted from each file by the abstraction tool, assigned a study code and scrutinized. It was then entered into a protected database by the principal investigator for subsequent data cleaning and analysis performed on SPSS version 23 with the help of a statistician.

Categorical variables such as comorbid conditions, sex and tacrolimus metabolising status are presented in tables as proportions and percentages on frequency tables. Pearson's chi square test is used for comparison of categorical variables. Continuous variables are presented as mean ± SD unless otherwise specified. ANOVA or Kruskal-Wallis is used to determine the difference in the mean eGFR

at one year between the strata formed by the C_0/D ratio. A trendline was used to display trends of graft function across the strata formed by C_0/D ratio. Statistical significance was set at $p \leq 0.05$.

3.7 Ethical Approval

The study was approved by the Department of Clinical Medicine and Therapeutics, Faculty of Health Sciences, University of Nairobi and the KNH/UoN ERC. The study was registered with the KNH Medical Research and Programs department. Access to medical records at the Renal Unit, was granted by the Health Record and Information department.

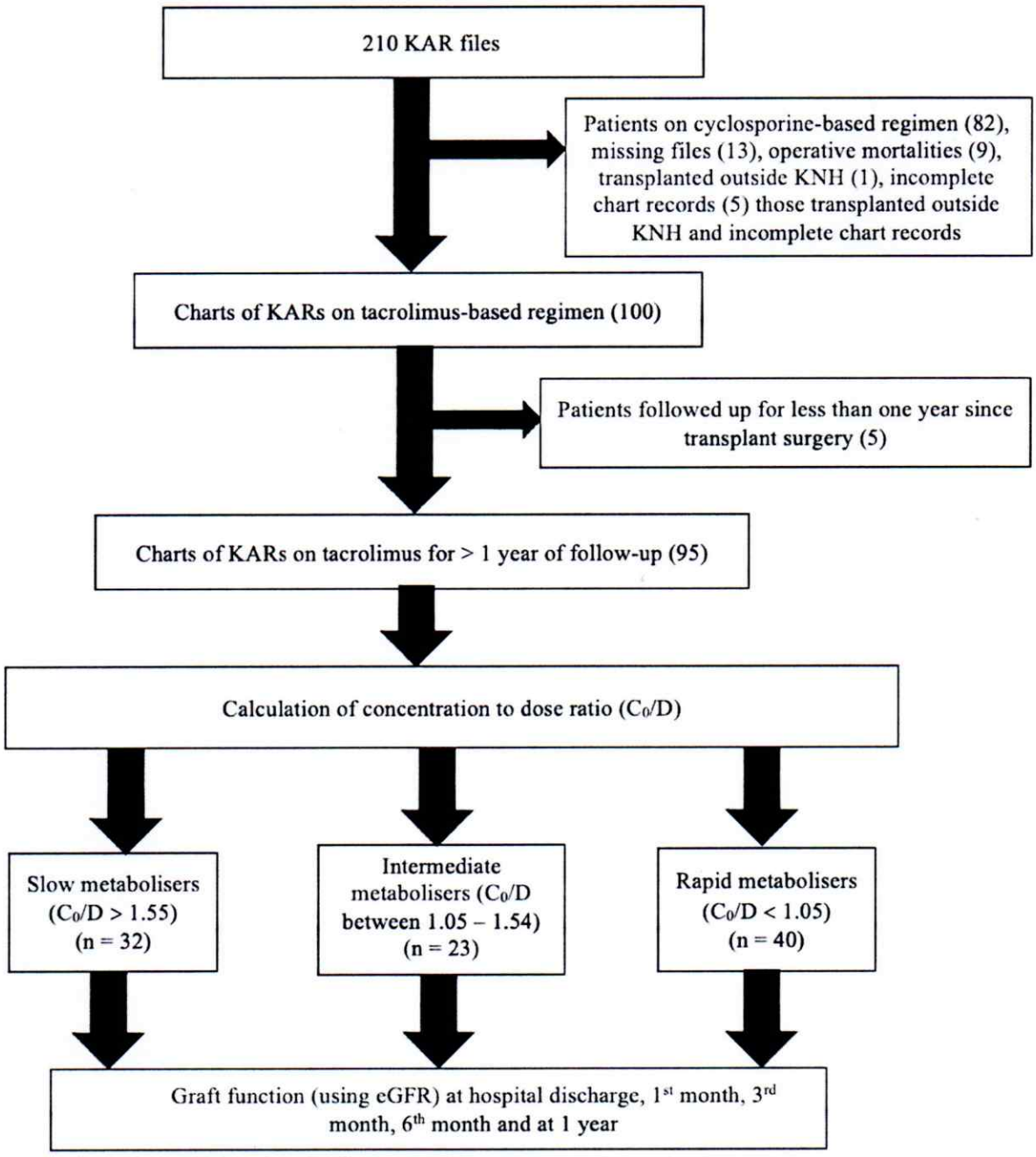
CHAPTER FOUR

4. RESULTS

4.1 Study recruitment

The study was undertaken between July 2022 and September 2022 at the Kenyatta National Hospital, Renal Unit. Data extraction was done at the transplant section with a decentralized records unit dedicated to kidney transplant recipients' medical records.

Two hundred and ten recipients' medical records were retrieved by the transplant co-ordinator and reviewed for study eligibility by the principal investigator and a research assistant. Of the 210 medical records, 110 were excluded because 82 recipients were on a cyclosporine-based regimen, 13 files were not located, 9 due to operative mortalities, 1 was transplanted at a site other than KNH and 5 had missing data on tacrolimus trough levels and corresponding tacrolimus dosages and frequency. Five recipient files were for patients who were on tacrolimus but had not completed one-year of follow-up. A total of 95 recipients with complete records were selected, coded, and included in the final analyses. The flowchart below describes the screening, recruitment, and analysis procedure.



Flowchart diagram 2 demonstrating the screening and recruitment results of the study.

4.2 Demographic profile of the study population

The eligible ninety-five patients were transplanted between 2010 and 2021 and among them, there were 67 males (70.5%). The mean age in years of the study population at the time of transplantation was 35.5 ± 13.6 years with a range of 10 to 60 years. More than half of kidney allograft recipients (57.1%) were hypertensive, 12.2% had diabetes mellitus and 43.9% had glomerulonephritis. The median dialysis vintage time was 18 months, with a minimum and maximum of 0 months and 75 months respectively. Three in every eight patients (37.5%) received induction therapy with basiliximab. Delayed allograft function was noted in 11.5% of the recipients (Table 3).

4.3 Medication-related characteristics of the study population

The average tacrolimus trough levels and tacrolimus and steroid dose of the population varied within the first year of kidney transplant surgery, with statistically significant variation at the discharge and within one, three, six and twelve months. Conversely, allograft function did not demonstrate significant variation across the time periods as demonstrated in table 2.

Table 2. Average tacrolimus trough and dose, steroid dose and allograft function among kidney allograft recipients taking tacrolimus at selected time-points within the first year of transplantation at the Kenyatta National Hospital.

Variable	Discharge	1 month	3 months	6 months	1 year	p-value
Tacrolimus trough levels (ng/ml)(mean(SD))	8.2 (3.9)	8.5 (4.4)	9.0 (3.7)	8.8 (4.5)	8.3 (3.9)	0.040
Tacrolimus dose (mg per day)(mean(SD))	8.1 (2.7)	7.7 (2.7)	7.2 (2.9)	6.6 (2.8)	5.8 (2.6)	<0.001
Steroid dose (mg/day)(mean(SD))	17.2 (6.8)	15.2 (5.8)	11.3 (4.3)	8.7 (3.2)	6.5 (3.3)	<0.001
Median eGFR (mls/min/1.73m^2)	81.3 (30.1)	88.7 (35.4)	82.9 (29.0)	85.5 (41.3)	84.9 (35.6)	0.147

Table 3. Sociodemographic and clinical characteristics of kidney allograft recipients taking tacrolimus at the Kenyatta National Hospital

Variable	Frequency, n (%)
Recipients mean age in yrs (SD)	35.0 (13.3)
Min-Max	10-60
Sex of recipient	
Female	28 (29.5)
Male	67 (70.5)
BMI recipient (kg/m^2)	
< 18.4	20 (21.1)
18.5 – 24.9	48 (50.5)
25 - 29.9	17 (17.9)
>30	1 (1.1)
Not documented	9 (9.5)
Dialysis vintage (months)	
Median	19.0
Min	0
Max	75
Pre-transplant co-morbidities	
Diabetes Mellitus	12 (12.6)
Hypertension	51 (53.7)
Glomerulonephritides	42 (44.2)
PCKD	5 (5.3)
***Others**	12 (12.6)
Obstructive uropathy	4 (4.2)
SLE	2 (2.1)
Induction therapy (Basiliximab)	
Yes	35 (37.5)
No	60 (62.5)
Delayed allograft function	
Yes	11 (11.6)
No	84 (88.4)

Others* - Focal Segmental Glomerulosclerosis, Mixed Connective Tissue Disease, Nephrotic Syndrome, Primary hyperoxaluria, NSAID nephropathy, Renal osteodystrophy, Alport Syndrome, Steroid Resistant Nephrotic Syndrome

4.4 Proportion of rapid, intermediate, and slow metabolisers

Forty (42.1%) kidney allograft recipients were fast metabolisers, with the lowest average C_0/D ratio of 0.79 compared to 1.17 and 2.12 of intermediate and slow metabolisers respectively. Twenty-three recipient files' (24.2%) were for intermediate metabolisers while the remainder (33.7%) consisted of slow metabolisers. A statistically significant difference in the steroid dose requirement was noted (p-value = 0.014) among the three metaboliser groups. Slow metabolisers demonstrated the highest median graft function (88.6 mls/min/1.73m^2) at the first year among the three groups although no statistically significant difference was noted (p value of 0.750).

Table 4. Frequency of tacrolimus metabolic phenotypes by C_0/D ratio among kidney allograft recipients at the Kenyatta National Hospital

Phenotype (C_0/D ratio)	Frequency, n (%)
Rapid tacrolimus metabolisers (RTM) (<1.05)	40 (42.1)
Intermediate tacrolimus metabolisers (ITM) (1.05 to 1.54)	23 (24.2)
Slow tacrolimus metabolisers (STM) (>1.55)	32 (33.7)

Table 5. Average C_0/D ratio, tacrolimus trough and dose, steroid dose, and allograft function among kidney allograft recipients as rapid, intermediate, and slow tacrolimus metabolisers at the Kenyatta National Hospital

Variable	RTM (n = 40)	ITM (n = 23)	STM (n = 32)	p-value
Average C_0/D ratio	0.79	1.17	2.12	**<0.001**
Mean tacrolimus trough (SD) (ng/ml)	7.0 (1.5)	8.2 (2.0)	10.8 (3.3)	**<0.001**
Mean tacrolimus dose (SD) (mg/day)	8.8 (2.0)	7.0 (1.5)	5.1 (1.5)	**<0.001**
Mean steroid dose (SD) (mg/day)	13.0 (3.3)	11.3 (3.5)	10.6 (3.5)	**0.014**
Median eGFR at 1year (IQR) (mls/min/1.73m^2)	83.4 (69.2 – 98.9)	82.1 (63.0 – 106.3)	88.6 (59.5 – 116.12)	**0.750**

4.5 Sociodemographic profile of the study population by metabolic phenotype(s)

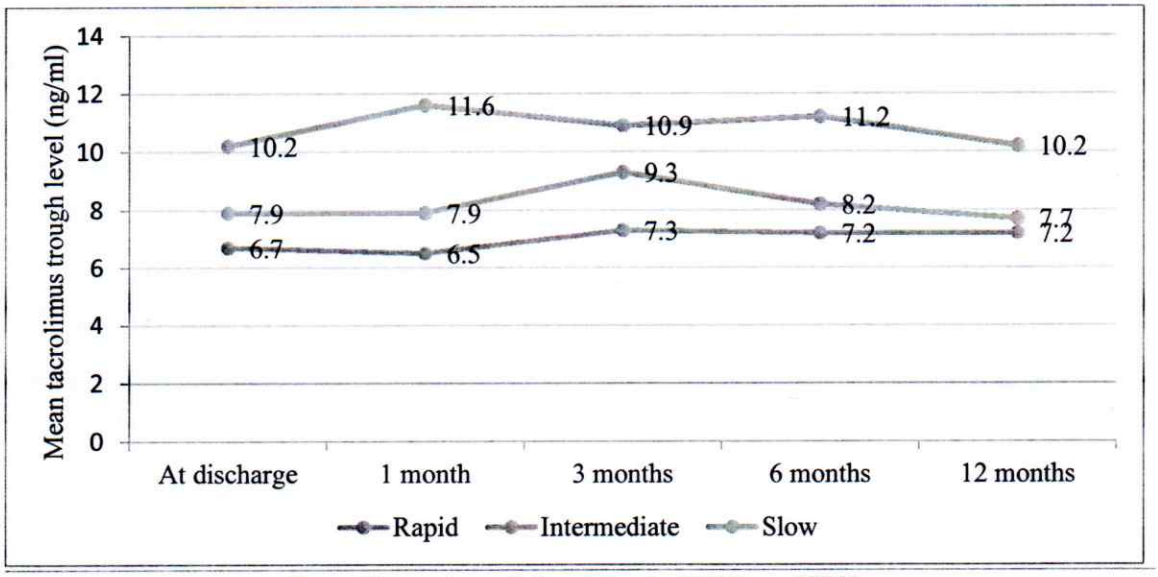
Forty (42.1%) kidney allograft recipient population taking tacrolimus consisted of rapid metabolisers, while 33.7% and 24.2% were slow and intermediate metabolisers respectively (table 3). The average age of rapid metabolisers was 33 years (±11.8). This was 4 years younger than the slow and intermediate metabolisers, who both had a mean age of 37 years, with a standard deviation of 14.5 and 14.4 years respectively (p value of 0.308). One in every two female patients were rapid metabolisers compared to 38% of the male population while approximately a third (34%) of both the female and male population were slow metabolisers. All patients within the three groups had similar dialysis vintage times as depicted in table 6. No statistically significant difference was noted among the sociodemographic profiles of the three metabolic phenotypes (table 6).

4.6 Medication-related characteristics of the study population by metabolic phenotype(s)

Tacrolimus trough concentrations were lower in the rapid metabolisers, with a mean of 7.0ng/ml (± 1.5) compared to the slow metabolisers, who had an average of 10.8ng/ml (± 3.3)(figure 2). This difference was statistically significant and consistent within the first year of kidney transplantation (figure 3). Conversely, slow metabolisers had a lower average total daily tacrolimus dose (5.1mg per day) compared to 8.8mg per day of tacrolimus within the rapid metabolisers group as depicted on figure 4. The difference was statistically significant, and the trend was consistent through the first year of kidney transplantation. Slow metabolisers demonstrated a 50% reduction in total tacrolimus daily dose from discharge to one year while intermediate and rapid metabolisers had a 20% decrease in the total tacrolimus daily dose (figure 4). The average daily steroid dose required was noted to be higher in the rapid metabolisers compared to the intermediate and slow metabolisers (p value – 0.014). The difference in the steroid dose requirements among rapid, intermediate, and slow tacrolimus metabolisers was statistically significant at three months and at one year as depicted in table 7.

Table 6. Selected demographic and clinical characteristics of rapid, intermediate, and slow tacrolimus metabolisers among kidney allograft recipients at the Kenyatta National Hospital

Variable	RTM (n = 40)	ITM (n= 23)	STM (n = 32)	p-value
Recipient age (yrs.)				
Mean (SD)	33.0 (11.8)	36.3 (14.4)	36.6 (14.5)	**0.456**
Sex of recipient				
Female	15 (37.5)	4 (17.4)	9 (28.1)	
Male	25 (62.5)	19 (82.6)	23 (71.9)	**0.237**
Dialysis vintage (months)				
Median	18	19	18.5	
Min	4	6	1	
Max	67	59	75	**0.831**
BMI recipient (kg/m^2)				
< 18.4	8 (21.1)	6 (26.0)	12 (37.5)	
18.5 – 24.9	23 (60.5)	10 (43.5)	15 (46.9)	
25 – 29.9	8 (18.4)	7 (30.4)	5 (15.6)	**0.686**
Co-morbidities				
Diabetes	2 (5.0)	3 (13.0)	7 (21.9)	**0.110**
Hypertension	21 (52.5)	13 (56.5)	17 (53.1)	**0.951**
Glomerulonephritis	21 (52.5)	9 (39.1)	12 (37.5)	**0.379**
PCKD	2 (5.0)	2 (8.7)	1 (3.1)	**0.721**
***Others**	3 (7.5)	4 (17.4)	5 (15.6)	**0.457**
Obstructive uropathy	1 (2.5)	2 (8.7)	1 (3.1)	**0.555**
SLE	1 (2.5)	0	1 (3.1)	**1.000**
Induction therapy with basiliximab				
Yes	14 (35.9)	9 (39.1)	12 (37.5)	
No	25 (62.5)	12 (52.2)	19 (59.4)	**0.320**
Delayed allograft function				
Yes	6 (15.0)	3 (13.0)	2 (6.2)	
No	34 (85.0)	20 (87.0)	30 (93.8)	**0.557**



Tacrolimus trough level (ng/ml) (Mean (SD))

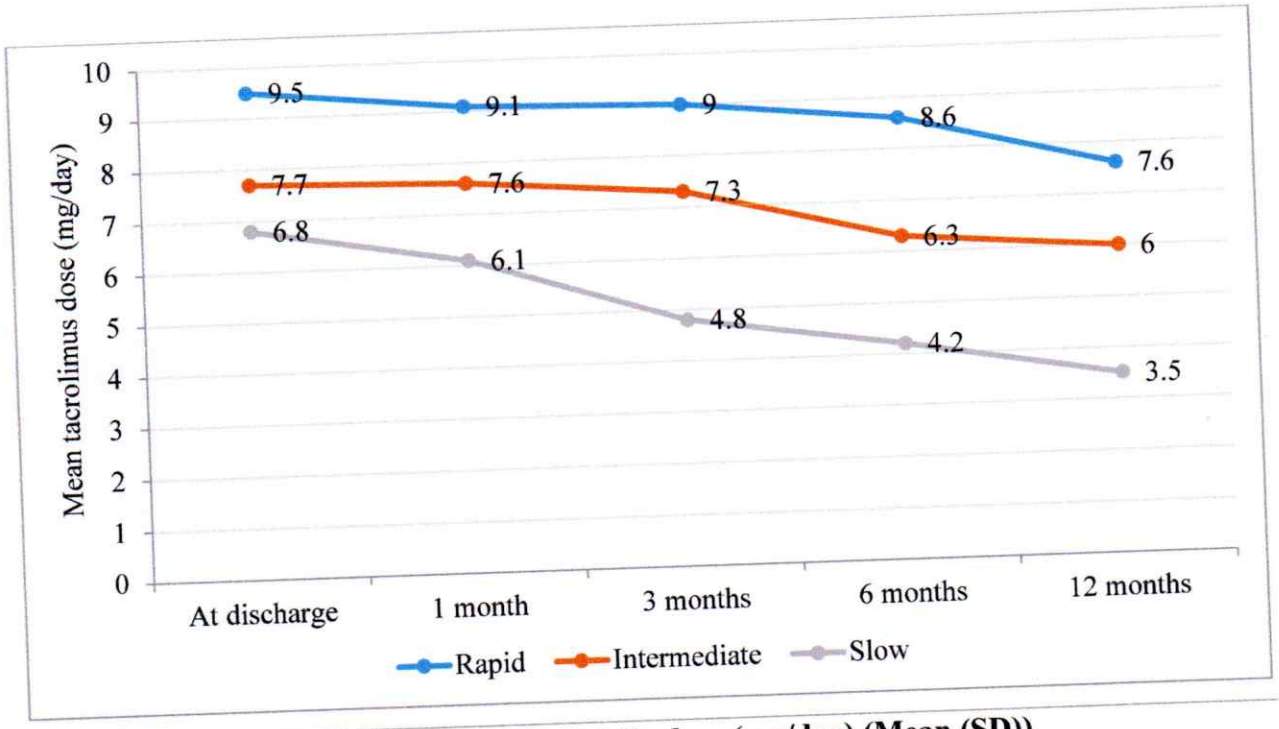
Selected time-points	RTM (n = 40)	ITM (n = 23)	STM (n = 32)	p-value
At discharge	6.7 (2.5)	7.9 (3.4)	10.2 (4.8)	**0.001**
1 month	6.5 (2.1)	7.9 (3.4)	11.6 (5.4)	**<0.001**
3 months	7.3 (2.3)	9.3 (3.0)	10.9 (4.9)	**<0.001**
6 months	7.2 (3.0)	8.2 (3.1)	11.2 (5.7)	**0.001**
12 months	7.2 (2.4)	7.7 (2.7)	10.2 (5.3)	**0.003**
Average	7.0 (1.5)	8.2 (2.0)	10.8 (3.3)	**<0.001**

Figure 3. Trendlines and a table demonstrating the mean tacrolimus trough concentration (ng/ml) within the first year of kidney transplantation (at hospital discharge, one month, three months, six months and at one year) among kidney allograft recipients as rapid, intermediate, and slow metabolisers of tacrolimus.

4.7 Trends of allograft function and metabolic phenotype(s) of the study population

Allograft function was assessed by the Modification of Diet in Renal Diseases (MDRD-4) equation. The median eGFR among all three metabolic phenotype was above 60mls/min/1.73m^2, demonstrating good allograft function throughout the first year of transplantation (table 8). Slow metabolisers demonstrated a lower eGFR (79.8mls/min/1.73m^2) upon discharge compared to rapid and intermediate metabolisers, 80.5mls/min/1.73m^2 and 85.3mls/min/1.73m^2 respectively. However, their average

eGFR steadily rose throughout the first year of kidney transplantation. Intermediate metabolisers had the highest recorded eGFR (93.2 mls/min/1.73m^2) in the 1st month, thereafter, a 13% decline was noted at the end of the first year of kidney transplantation to the lowest eGFR at one year of kidney transplantation as depicted in figure 5.



Tacrolimus total daily dose (mg/day) (Mean (SD))

Selected timepoints	RTM (n = 40)	ITM (n = 23)	STM (n = 32)	p-value
At discharge	9.5 (2.7)	7.7 (2.2)	6.8 (2.4)	**<0.001**
1 month	9.1 (2.7)	7.6 (2.0)	6.1 (2.2)	**<0.001**
3 months	9.0 (2.4)	7.3 (2.1)	4.8 (2.4)	**<0.001**
6 months	8.6 (2.4)	6.3 (1.7)	4.2 (2.0)	**<0.001**
12 months	7.6 (1.9)	6.0 (1.7)	3.5 (2.0)	**<0.001**
Average	8.8 (2.0)	7.0 (1.5)	5.1 (1.5)	**<0.001**

Figure 4. Trendlines and a table demonstrating the average tacrolimus dose at selected time-points within the first year of kidney transplantation among kidney allograft recipients as rapid, intermediate, and slow metabolisers of tacrolimus.

Table 7. Steroid dose requirements among kidney allograft recipients as rapid, intermediate, and slow metabolisers at selected time points within the first year of kidney transplantation

	Steroid dose (mg/day) (Mean (SD))			
Selected timepoints	**RTM (n = 40)**	**ITM (n = 23)**	**STM (n = 32)**	**p-value**
At discharge	18.7 (6.6)	16.0 (6.5)	16.1 (7.2)	**0.168**
1 month	16.9 (5.0)	14.2 (5.8)	13.8 (6.1)	**0.036**
3 months	12.6 (4.0)	10.9 (4.3)	10.1 (4.4)	**0.045**
6 months	9.4 (3.5)	8.6 (2.5)	8.0 (3.0)	**0.162**
12 months	7.2 (3.4)	7.2 (4.5)	5.3 (1.2)	**0.026**
Average	13.0 (3.3)	11.3 (3.5)	10.7 (3.5)	**0.014**

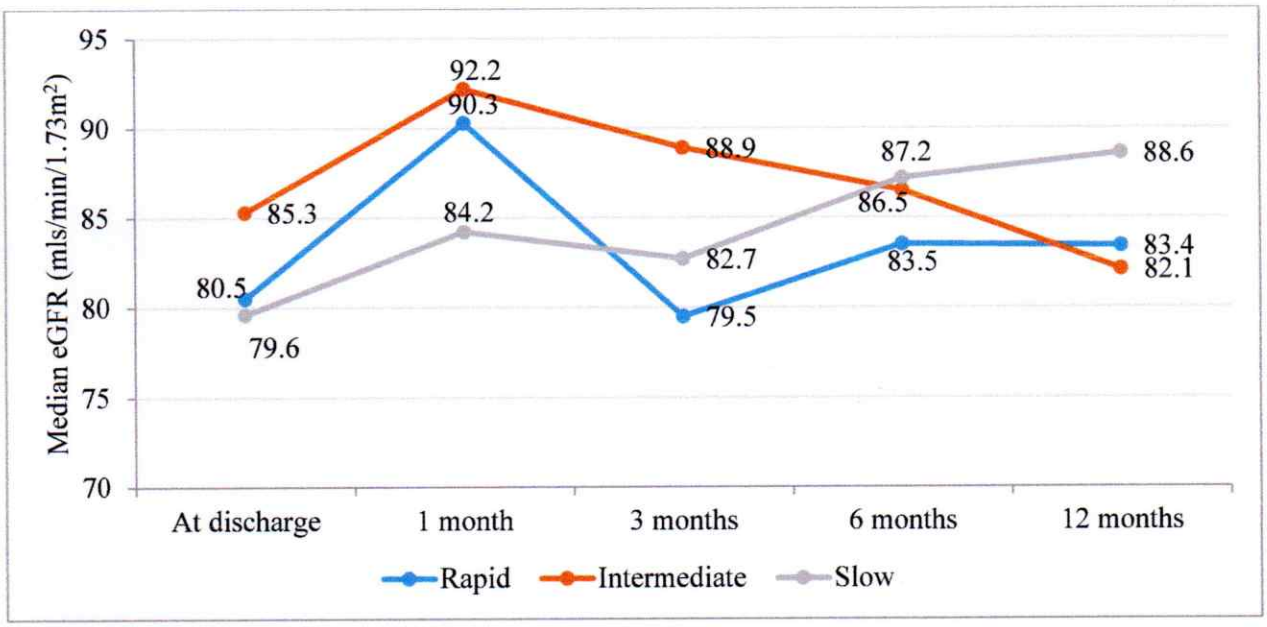


Figure 5. Trendlines demonstrating the average allograft function as measured by eGFR at selected time-points within the first year of transplantation among kidney allograft recipients as rapid, intermediate, and slow tacrolimus metabolisers.

Table 8. Allograft function expressed as calculated eGFR among kidney allograft recipients as rapid, intermediate, and slow metabolisers at selected time points within the first year of kidney transplantation.

	Median eGFR(mls/min/1.73m^2)			
Selected timepoints	**RTM (n = 40)**	**ITM (n = 23)**	**STM (n = 32)**	**p-value**
At discharge	80.5	85.3	79.6	**0.788**
IQR	(60.6 – 98.0)	(63.2 – 99.6)	(57.6 – 91.4)	
1 month	90.3	92.2	84.2	**0.655**
IQR	(67.5 – 102.3)	(74.2 – 113.1)	(67.3 – 97.9)	
3 months	79.5	88.9	82.7	**0.579**
IQR	(68.4 – 85.8)	(66.2 – 105.6)	(63.7 – 101.5)	
6 months	83.5	86.5	87.2	**0.933**
IQR	(68.0 – 92.0)	(68.5 – 91.1)	(62.2 – 138.0)	
12 months	83.4	82.1	88.6	**0.750**
IQR	(69.2 – 98.9)	(63.0 – 106.3)	(59.5 – 116.12)	

CHAPTER FIVE

DISCUSSION

Understanding pharmacogenetic mechanisms by which kidney allograft recipients metabolise tacrolimus in the maintenance phase of immunosuppressive treatment is an important step towards achieving precision medicine. Herein, we present the distribution of tacrolimus metabolic phenotypes by calculating tacrolimus dose-adjusted trough concentration (C_0/D ratio), a key genotype predictor (9) and prognostic indicator of graft survival(11), among kidney allograft recipients taking tacrolimus at the Kenyatta National Hospital. The study found that 42% of kidney allograft recipients taking tacrolimus are rapid metabolisers (C_0/D ratio < 1.05), CYP3A5*1*1 carriers, while 33.7% and 24.2% are slow and intermediate metabolisers (C_0/D ratio > 1.05) respectively. A similar trend was noted in a study of multiracial patients in South Africa where rapid tacrolimus metabolisers were 72% of the 43 patients and all black patients in that study were reported to be rapid metabolisers(54). This figure is notably higher as rapid (CYP3A5*1*1 carriers) and intermediate (CYP3A5*1/*3 carriers) were aggregated and classified as rapid metabolisers due to the common CYP3A5*1 allele. In Southeast Asia and North Africa, the frequency of rapid metabolisers was found to be 5.9% and 15% respectively while that of slow metabolisers was shown to be 70% and 85% respectively(55,56). Both studies included individuals carrying one or both CYP3A5*3 alleles (intermediate and slow metabolisers) in the slow metabolisers group thus accounting for the higher proportion. Slow and intermediate metabolisers in black ethnic groups were demonstrated to be carriers of one or both CYP3A5*3, CYP3A5*6, and CYP3A5*7 alleles(57), possibly accounting for this proportion in our setup. This study found an even distribution of CYP3A5 expressors and non-expressors in our population, possibly corroborating with the diversity of the CYP3A5 gene in the African setting(58).

The tacrolimus dose required to achieve therapeutic range tacrolimus trough level is higher in rapid metabolisers (CYP3A5*1/*1) due to increased CYP3A5 enzyme activity. This study found that rapid metabolisers required a 42% higher average total tacrolimus daily dose of 8.8mg compared to 5.1mg for the slow metabolisers, a difference that was statistically significant throughout the first year of transplant (p value of < 0.001). Tacrolimus trough concentrations were 35% higher in the slow metabolisers group compared to the rapid metabolisers. These findings compare well with data from diverse racial groups worldwide(59). There was a notable reduction in steroid dose requirement throughout the first year within all groups. We found the lowest average steroid dose requirement among slow tacrolimus metabolisers (10.7mg) compared to intermediate (11.3mg) and fast (13.0 mg)

tacrolimus metabolisers, a difference that was statistically significant. Carriers of at least one CYP3A5*3 allele (intermediate and slow tacrolimus metabolisers) have been shown to require a lower steroid dose to maintain therapeutic range tacrolimus trough concentration. Steroids induce expression of CYP3A through pregnane X receptor thus increasing tacrolimus metabolism, therefore steroid tapering lowers this stimulatory effect(38,60). This possibly explains the difference in steroid requirement between the slow, intermediate, and fast metabolisers in this setup.

This study found lower allograft function among rapid and intermediate metabolisers compared to slow tacrolimus metabolisers. Patients with lower C_0/D ratios demonstrate higher fluctuations in tacrolimus drug levels between subsequent doses therefore susceptible to tacrolimus nephrotoxicity due to high peak tacrolimus concentrations(61). High tacrolimus intra-patient variability leads to alternating periods of immune-activation with subclinical rejection and tacrolimus-mediated toxicity and target-organ damage(62). Intra-patient variability, one of the predictors of reduced graft function(63,64), appears to be higher in rapid and intermediate metabolisers (carriers of at least one CYP3A5*1 allele)(65). However, whether the genotype and subsequent metabolic phenotype directly influences intra-patient tacrolimus variability is yet to be fully determined. This is partly due to varied definitions of intra-individual variability(66–68). Although this study did not set out to determine intra-patient variability in our population, the phenomenon partly explains the lower eGFR among the rapid and intermediate metabolisers compared to the slow metabolisers.

A positive correlation between C_0/D ratio, genotype status and graft function has been previously demonstrated (53,69). The United Network for Organ Sharing (UNOS) in 1994 showed that black recipients had inferior graft outcomes due to donor-recipient HLA mismatches, socioeconomic differences and impaired access to healthcare services(70). Ten years later, with genotype testing, poor outcomes in African-Americans were also attributed to higher tacrolimus dose requirements due to fast metabolism(71). Genotypic testing partly elaborated on an important observation in kidney transplantation outcomes. Although the difference was not statistically significant in our study, one-year eGFR was lower in fast metabolisers compared to slow metabolisers. Potential reasons could be higher rates of subclinical rejection episodes as observed in black recipients(72) and tacrolimus-mediated nephrotoxicity, as noted in patients with lower C_0/D ratio(73). In this study, it was noted that intermediate metabolisers had the lowest eGFR at the end of the first year of kidney transplantation. The hypothesis is that a discrepancy between the observed phenotype and an individual's genotype-based diagnosis may exist, a phenomenon known as phenoconversion. This could be a result of non-genetic factors such as food-drug interactions, drug-drug interactions, and inter-current illnesses. In

this study, it may have been due to a prolonged dialysis vintage time due to chronic uraemic exposure, one factor known to increase the C_0/D ratio in kidney transplant recipients therefore phenoconverting fast metabolisers into intermediate metabolisers(74). Alternatively, unique CYP3A5 alleles found in African Americans may have variable levels of metabolic influence on tacrolimus dose(57,75). Altogether, additional factors not deducible from this study's limited design play a significant role.

The study found that more than half (54%) of the female kidney transplant recipients were fast tacrolimus metabolisers compared to 37% of the male recipients. Consistent with previously published literature, females, especially of black race, demonstrate rapid tacrolimus clearance compared to the male gender and persons of white race(76). Several other factors that determine candidacy for kidney transplantation such as socioeconomic status must have influenced our study population as a determinant of access to kidney transplantation. The average age of rapid tacrolimus metabolisers in this study was 33 years, the lowest among all three groups (37 years for both intermediate and slow metabolisers). This age difference was not statistically significant possibly due to homogeneity of our population. Previous research demonstrated that rapid metabolisers tend to be younger (< 50 years of age), as in our population, and this age effect appears to be independent of the recipient's genotype and the type of solid organ transplantation(77–79). Known predictors of slow metabolisers are male gender, older age (>60 years of age) and a higher BMI(80). Of note, individuals with a BMI of more than 24.9kg/m^2 are phenotypically slow metabolisers and as a result, demonstrate higher tacrolimus trough concentration. This effect also persists despite the genotype status(81). In this study, more than half of the individuals were of optimal BMI and of the twenty individuals with a BMI greater than 24.9kg/m^2, 40% and 25% were rapid and slow tacrolimus metabolisers respectively. These findings did not replicate the BMI-effect, likely due to the small number of individuals in that bracket.

Conclusions:

The study results suggest that:

i. Forty-two percent of kidney transplant recipients in our population are rapid metabolisers with a high tacrolimus dose requirement.

ii. Rapid metabolisers are at risk of deteriorating allograft function within the first year compared to intermediate and slow tacrolimus metabolisers, especially if dose adjustment is solely guided by tacrolimus trough concentration. It is plausible that phenoconversion may play a role in influencing a patient's metabolic phenotype and determine the subsequent tacrolimus dose requirement.

Limitations:

i. This is a single centre study with a small sample size thus affecting generalisability of the findings.

ii. One-year of follow up is short compared to studies that compare 2-year and 5-year outcomes.

iii. The study did not account for the effect of co-medications that may induce and/or inhibit drug metabolising enzymes and transporters (DMETs) involved in tacrolimus disposition.

iv. This may affect the tacrolimus trough level reported, interfere with the C_0/D ratio thus resulting in metabolic phenotype reclassification. The study did not explore the role of other enzymes (CYP3A4) and transporters (ABCB1) that play a role in tacrolimus pharmacokinetics.

v. The effect of different formulations of tacrolimus was also not explored in this study.

Strengths:

The study forms a basis for future pharmacogenetic studies, not only locally but also in East Africa, leveraging on genetic information for precision medicine to improve long-term outcomes, within organ transplantation clinical practice and beyond.

Recommendations:

i. A prospective pharmacokinetic study to determine the full concentration-time profile of tacrolimus in kidney allograft recipients and correlate this information with allograft function beyond one year and important transplant-related outcomes such as rejection.

ii. Genotypic testing to definitively determine the status of clinically relevant drug-metabolising enzymes and transporters.

REFERENCES

1. Fuehner T, Benden C, Gottlieb J. Initial immunosuppression and managing rejection. Intensive Care Med. 2019 Mar 13;45(3):388–90.

2. Neuwirt H, Rudnicki M, Schratzberger P, Pirklbauer M, Kronbichler A, Mayer G. Immunosuppression after renal transplantation. memo. 2019 Sep;12(3):216–21.

3. Liu J yu, You R xu, Guo M, Zeng L, Zhou P, Zhu L, et al. Tacrolimus Versus Cyclosporine as Primary Immunosuppressant After Renal Transplantation: A Meta-Analysis and Economics Evaluation. American Journal of Therapeutics. 2016;15.

4. Gonwa T, Johnson C, Ahsan N, Alfrey EJ, Halloran P, Stegall M, et al. Randomized trial of tacrolimus + mycophenolate mofetil or azathioprine versus cyclosporine + mycophenolate mofetil after cadaveric kidney transplantation: results at three years: Transplantation. 2003 Jun;75(12):2048–53.

5. Brunet M, van Gelder T, Åsberg A, Haufroid V, Hesselink DA, Langman L, et al. Therapeutic Drug Monitoring of Tacrolimus-Personalized Therapy: Second Consensus Report. Therapeutic Drug Monitoring. 2019 Jun;41(3):261–307.

6. Birdwell KA, Grady B, Choi L, Xu H, Bian A, Denny JC, et al. The use of a DNA biobank linked to electronic medical records to characterize pharmacogenomic predictors of tacrolimus dose requirement in kidney transplant recipients. Pharmacogenetics and Genomics. 2012 Jan;22(1):32–42.

7. Kalebi A, Ngari C. POS-737 Immunosuppressant Therapeutic Drug Monitoring Of Tacrolimus And Cyclosporine Levels At An Independent Central Laboratory In Kenya. Kidney International Reports. 2021 Apr;6(4):S321.

8. Song T, Yin S, Jiang Y, Huang Z, Liu J, Wang Z, et al. Increasing Time in Therapeutic Range of Tacrolimus in the First Year Predicts Better Outcomes in Living-Donor Kidney Transplantation. Front Immunol. 2019 Dec 20;10:2912.

9. Birdwell K, Decker B, Barbarino J, Peterson J, Stein C, Sadee W, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 Genotype and Tacrolimus Dosing. Clin Pharmacol Ther. 2015 Jul;98(1):19–24.

10. Bartlett FE, Carthon CE, Hagopian JC, Horwedel TA, January SE, Malone A. Tacrolimus Concentration-to-Dose Ratios in Kidney Transplant Recipients and Relationship to Clinical Outcomes. Pharmacotherapy. 2019 Aug;39(8):827–36.

11. Jouve T, Fonrose X, Noble J, Janbon B, Fiard G, Malvezzi P, et al. The TOMATO Study (Tacrolimus Metabolization in Kidney Transplantation): Impact of the Concentration–Dose Ratio on Death-censored Graft Survival. Transplantation. 2020 Jun;104(6):1263–71.

12. Kahan BD. Toward a Rational Design of Clinical Trials of Immunosuppressive Agents in Transplantation. Immunol Rev. 1993 Dec;136(1):29–49.

13. Ekberg H, Vítko Š, Margreiter R, Frei U, Halloran PF. Reduced Exposure to Calcineurin Inhibitors in Renal Transplantation. n engl j med. 2007;14.

14. Liu J, Farmer JD, Lane WS, Friedman J, Weissman I, Schreiber SL. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell. 1991 Aug;66(4):807–15.

15. Erdman J, Wolfram J, Nimke D, Croy R, Wang X, Weaver T, et al. Lung Transplant Outcomes in Adults in the United States: Retrospective Cohort Study Using Real-world Evidence from the SRTR. Transplantation. 2022 Jun;106(6):1233–42.

16. Satoh S, Tada H, Tachiki Y, Tsuchiya N, Shimoda N, Akao T, et al. Chrono and clinical pharmacokinetic study of tacrolimus in continuous intravenous administration. Int J Urol. 2001 Jul;8(7):353–8.

17. Savic, M.; Rossi, A.; Vella, J. Sublingual Tacrolimus Is a Safe, Cost-Effective Alternative to Intravenous Tacrolimus in Kidney Transplant Recipients., Transplantation: July 15, 2014 - Volume 98 - Issue - p 682.

18. Hougardy JM, Broeders N, Kianda M, Massart A, Madhoun P, Le Moine A, et al. Conversion from Prograf to Advagraf Among Kidney Transplant Recipients Results in Sustained Decrease in Tacrolimus Exposure. Transplantation. 2011 Mar 15;91(5):566–9.

19. Bekersky I, Dressler D, Mekki Q. Effect of time of meal consumption on bioavailability of a single oral 5 mg tacrolimus dose. J Clin Pharmacol. 2001 Mar;41(3):289-97.

20. Thorn M, Finnstrom N, Lundgren S, Rane A, Loof L. Cytochromes P450 and MDR1 mRNA expression along the human gastrointestinal tract. Br J Clin Pharmacol. 2005 Jul;60(1):54–60.

21. Bekersky I, Dressler D, Mekki QA. Effect of Low- and High-Fat Meals on Tacrolimus Absorption following 5 mg Single Oral Doses to Healthy Human Subjects. The Journal of Clinical Pharmacology. 2001 Feb;41(2):176–82.

22. Homma M, Itagaki F, Yuzawa K, Fukao K, Kohda Y. Effects of Lansoprazole and Rabeprazole On Tacrolimus Blood Concentration: Case Of A Renal Transplant Recipient With Cyp2c19 Gene Mutation: Transplantation. 2002 Jan;73(2):303–4.

23. Hosohata K, Masuda S, Ogura Y, Oike F, Takada Y, Katsura T, et al. Interaction between Tacrolimus and Lansoprazole, but not Rabeprazole in Living-Donor Liver Transplant Patients with Defects of CYP2C19 and CYP3A5. Drug Metabolism and Pharmacokinetics. 2008;23(2):134–8.

24. Bremer SCB, Reinhardt L, Sobotta M, Hasselluhn MC, Lorf T, Ellenrieder V, et al. Pantoprazole Does not Affect Serum Trough Levels of Tacrolimus and Everolimus in Liver Transplant Recipients. Front Med. 2018 Nov 19;5:320.

25. Tsunashima D, Kawamura A, Murakami M, Sawamoto T, Undre N, Brown M, et al. Assessment of Tacrolimus Absorption from the Human Intestinal Tract: Open-Label, Randomized, 4-Way Crossover Study. Clinical Therapeutics. 2014 May;36(5):748–59.

26. Sato K, Amada N, Sato T, Miura S, Ohashi Y, Sekiguchi S, et al. Severe elevations of FK506 blood concentration due to diarrhea in renal transplant recipients. Clin Transplant. 2004 Oct;18(5):585–90.

27. Nakamura A, Amada N, Haga I, Tokodai K, Kashiwadate T. Effects of Elevated Tacrolimus Trough Levels in Association with Infectious Enteritis on Graft Function in Renal Transplant Recipients. Transplantation Proceedings. 2014 Mar;46(2):592–4.

28. Klomp SD, Arends EJ, Teng YKO, Swen JJ. Increased Tacrolimus Exposure in Kidney Transplant Recipients With COVID-19: Inflammation-Driven Downregulation of Metabolism as a Potential Mechanism. Transplant International. 2022;35:9.

29. Guo Y, Crnkovic CM, Won KJ, Yang X, Lee JR, Orjala J, et al. Commensal Gut Bacteria Convert the Immunosuppressant Tacrolimus to Less Potent Metabolites. Drug Metab Dispos. 2019 Mar;47(3):194–202.

30. Piletta-Zanin A, De Mul A, Rock N, Lescuyer P, Samer CF, Rodieux F. Case Report: Low Hematocrit Leading to Tacrolimus Toxicity. Front Pharmacol. 2021 Aug 16;12:717148.

31. 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 Jun;59(6):771–80.

32. Capron A, Mourad M, De Meyer M, De Pauw L, Eddour DC, Latinne D, et al. CYP3A5 and ABCB1 polymorphisms influence tacrolimus concentrations in peripheral blood mononuclear cells after renal transplantation. Pharmacogenomics. 2010 May;11(5):703–14.

33. Yokogawa K, Takahashi M, Tamai I, Konishi H, Nomura M, Moritani S, Miyamoto K, Tsuji A. P-glycoprotein-dependent disposition kinetics of tacrolimus: studies in mdr1a knockout mice. Pharm Res. 1999 Aug;16(8):1213-8.

34. Shiraga T, Matsuda H, Nagase K, Iwasaki K, Noda K, Yamazaki H, et al. Metabolism of FK506, a potent immunosuppressive agent, by cytochrome P450 3A enzymes in rat, dog and human liver microsomes. Biochemical Pharmacology. 1994 Feb;47(4):727–35.

35. Zegarska J, Hryniewiecka E, Zochowska D, Samborowska E, Jazwiec R, Borowiec A, et al. Tacrolimus Metabolite M-III May Have Nephrotoxic and Myelotoxic Effects and Increase the Incidence of Infections in Kidney Transplant Recipients. Transplantation Proceedings. 2016 Jun;48(5):1539–42.

36. Velicković-Radovanović R, Mikov M, Paunović G, Djordjević V, Stojanović M, Cvetković T, et al. Gender Differences in Pharmacokinetics of Tacrolimus and Their Clinical Significance in Kidney Transplant Recipients. Gender Medicine. 2011 Feb;8(1):23–31.

37. Rancic N, Dragojevic-Simic V, Vavic N, Kovacevic A, Segrt Z, Draskovic-Pavlovic B, et al. Tacrolimus concentration/dose ratio as a therapeutic drug monitoring strategy: The influence of gender and comedication. VSP. 2015;72(9):813–22.

38. Stifft F, van Kuijk SMJ, Bekers O, Christiaans MHL. Increase in tacrolimus exposure after steroid tapering is influenced by CYP3A5 and pregnane X receptor genetic polymorphisms in renal transplant recipients. Nephrology Dialysis Transplantation. 2018

39. Kim JH, Han N, Kim MG, Yun HY, Lee S, Bae E, et al. Increased Exposure of Tacrolimus by Co-administered Mycophenolate Mofetil: Population Pharmacokinetic Analysis in Healthy Volunteers. Sci Rep. 2018 Dec;8(1):1687.

40. Koch I, Weil R, Wolbold R, Brockmöller J, Hustert E, Burk O, Nuessler A, Neuhaus P, Eichelbaum M, Zanger U, Wojnowski L. Interindividual variability and tissue-specificity in the expression of cytochrome P450 3A mRNA. Drug Metab Dispos. 2002 Oct;30(10):1108-14. doi: 10.1124/dmd.30.10.1108. PMID: 12228187.

41. Metalidis C, Lerut E, Naesens M, Kuypers DRJ. Expression of CYP3A5 and P-glycoprotein in Renal Allografts with Histological Signs of Calcineurin Inhibitor Nephrotoxicity. Transplantation. 2011 May 27;91(10):1098–102.

42. Tapirdamaz Ö, Hesselink DA, el Bouazzaoui S, Azimpour M, Hansen B, van der Laan LJW, et al. Genetic variance in ABCB1 and CYP3A5 does not contribute toward the development of chronic kidney disease after liver transplantation. Pharmacogenetics and Genomics. 2014 Sep;24(9):427–35.

43. Christians U, Vinks AA, Langman LJ, Clarke W, Wallemacq P, van Gelder T, et al. Impact of Laboratory Practices on Interlaboratory Variability in Therapeutic Drug Monitoring of Immunosuppressive Drugs. Therapeutic Drug Monitoring. 2015 Dec;37(6):718–24.

44. Dubbelboer IR, Pohanka A, Said R, Rosenborg S, Beck O. Quantification of Tacrolimus and Three Demethylated Metabolites in Human Whole Blood Using LC–ESI–MS/MS. Therapeutic Drug Monitoring. 2012 Apr;34(2):134–42.

45. Tempestilli M, Di Stasio E, Basile MR, Elisei F, Antonini M, Ettorre GM, et al. Low Plasma Concentrations of Albumin Influence the Affinity Column–Mediated Immunoassay Method for the Measurement of Tacrolimus in Blood During the Early Period After Liver Transplantation. Therapeutic Drug Monitoring. 2013 Feb;35(1):96–100.

46. Wallemacq P, Goffinet JS, O'Morchoe S, Rosiere T, Maine GT, Labalette M, et al. Multi-site Analytical Evaluation of the Abbott ARCHITECT Tacrolimus Assay. Therapeutic Drug Monitoring. 2009 Apr;31(2):198–204.

47. Larpparisuth N, Pongnatcha T, Panprom P, Promraj R, Premasathian N, Vongwiwatana A. High Intrapatient Variability in Tacrolimus Exposure Calculated Over a Long Period Is Associated With De Novo Donor-Specific Antibody Development and/or Late Rejection in Thai Kidney Transplant Patients Receiving Concomitant CYP3A4/5 Inhibitors. Therapeutic Drug Monitoring. 2021 Oct;43(5):624–9.

48. Stefanović NZ, Cvetković TP, Jevtović-Stoimenov TM, Ignjatović AM, Paunović GJ, Veličković RM. Investigation of CYP 3A5 and ABCB1 gene polymorphisms in the long-term following renal transplantation: Effects on tacrolimus exposure and kidney function. Experimental and Therapeutic Medicine. 2015 Sep;10(3):1149–56.

49. Foroutan F, Friesen EL, Clark KE, Motaghi S, Zyla R, Lee Y, et al. Risk Factors for 1-Year Graft Loss After Kidney Transplantation: Systematic Review and Meta-Analysis. CJASN. 2019 Nov 7;14(11):1642–50.

50. Montero N, Codina S, Cruzado JM. Prediction scores for risk of allograft loss in patients receiving kidney transplants: nil satis nisi optimum. Clinical Kidney Journal. 2020 Oct 1;13(5):745–8.

51. Kabinga S, McLigeyo S, Ngigi J, Kibe E. POS-773 Calcineurin Inhibitors Dosage and Resultant Blood Trough Levels on Day Three And Seven In Kidney Transplant Recipents In Kenya: A Single Centre Observation. Kidney International Reports. 2022 Feb;7(2):S333.

52. for the DEKAF Investigators, Sanghavi K, Brundage RC, Miller MB, Schladt DP, Israni AK, et al. Genotype-guided tacrolimus dosing in African-American kidney transplant recipients. Pharmacogenomics J. 2017 Jan;17(1):61–8.

53. Thölking G, Fortmann C, Koch R, Gerth HU, Pabst D, Pavenstädt H, et al. The Tacrolimus Metabolism Rate Influences Renal Function after Kidney Transplantation. Bueno V, editor. PLoS ONE. 2014 Oct 23;9(10):e111128.

54. Muller WK, Dandara C, Manning K, Mhandire D, Ensor J, Barday Z, et al. CYP3A5 polymorphisms and their effects on tacrolimus exposure in an ethnically diverse South African renal transplant population. S Afr Med J. 2020 Jan 29;110(2):159.

55. Mendrinou E, Mashaly ME, Al Okily AM, Mohamed ME, Refaie AF, Elsawy EM, et al. CYP3A5 Gene-Guided Tacrolimus Treatment of Living-Donor Egyptian Kidney Transplanted Patients. Front Pharmacol. 2020 Aug 11;11:1218.

56. Xuan NT, Hop VQ, Kien TQ, Toan PQ, Thang LV, Binh HT, et al. Frequencies and Association of CYP3A5 Polymorphism with Tacrolimus Concentration Among Renal Transplant Recipients in Vietnam. Transplantation Proceedings. 2022 Sep;S0041134522005140.

57. Oetting WS, Schladt DP, Guan W, Miller MB, Remmel RP, Dorr C, et al. Genomewide Association Study of Tacrolimus Concentrations in African American Kidney Transplant Recipients Identifies Multiple CYP3A5 Alleles: TAC Troughs in AA Recipients. American Journal of Transplantation. 2016 Feb;16(2):574–82.

58. Bains RK, Kovacevic M, Plaster CA, Tarekegn A, Bekele E, Bradman NN, et al. Molecular diversity and population structure at the Cytochrome P450 3A5 gene in Africa. BMC Genet. 2013;14(1):34.

59. Mohamed ME, Schladt DP, Guan W, Wu B, Setten J, Keating BJ, et al. Tacrolimus troughs and genetic determinants of metabolism in kidney transplant recipients: A comparison of four ancestry groups. Am J Transplant. 2019 Oct;19(10):2795–804.

60. Anglicheau D. Pharmacokinetic interaction between corticosteroids and tacrolimus after renal transplantation. Nephrology Dialysis Transplantation. 2003 Nov 1;18(11):2409–14.

61. Thölking G, Schütte-Nütgen K, Schmitz J, Rovas A, Dahmen M, Bautz J, et al. A Low Tacrolimus Concentration/Dose Ratio Increases the Risk for the Development of Acute Calcineurin Inhibitor-Induced Nephrotoxicity. JCM. 2019 Oct 2;8(10):1586.

62. Shuker N, Shuker L, van Rosmalen J, Roodnat JI, Borra LCP, Weimar W, et al. A high intrapatient variability in tacrolimus exposure is associated with poor long-term outcome of kidney transplantation. Transpl Int. 2016 Nov;29(11):1158–67.

63. Süsal C, Döhler B. Late intra-patient tacrolimus trough level variability as a major problem in kidney transplantation: A Collaborative Transplant Study Report. Am J Transplant. 2019 Oct;19(10):2805–13.

64. Park Y, Lee H, Eum SH, Kim HD, Ko EJ, Yang CW, et al. Intrapatient Variability in Tacrolimus Trough Levels Over 2 Years Affects Long-Term Allograft Outcomes of Kidney Transplantation. Front Immunol. 2021 Sep 30;12:746013.

65. Ro H, Min SI, Yang J, Moon KC, Kim YS, Kim SJ, et al. Impact of Tacrolimus Intraindividual Variability and CYP3A5 Genetic Polymorphism on Acute Rejection in Kidney Transplantation. Therapeutic Drug Monitoring. 2012 Dec;34(6):680–5.

66. Pashaee N, Bouamar R, Hesselink DA, Roodnat JI, van Schaik RH, Weimar W, et al. CYP3A5 Genotype Is Not Related to the Intrapatient Variability of Tacrolimus Clearance. Therapeutic Drug Monitoring. 2011 Jun;33(3):369–71.

67. Spierings N, Holt DW, MacPhee IAM. CYP3A5 Genotype Had no Impact on Intrapatient Variability of Tacrolimus Clearance in Renal Transplant Recipients. Therapeutic Drug Monitoring. 2013 Jun;35(3):328–31.

68. Seibert SR, Schladt DP, Wu B, Guan W, Dorr C, Remmel RP, et al. Tacrolimus trough and dose intra-patient variability and CYP3A5 genotype: Effects on acute rejection and graft failure in European American and African American kidney transplant recipients. Clin Transplant. 2018 Dec;32(12):e13424.

69. van Gelder T, Meziyerh S, Swen JJ, de Vries APJ, Moes DJAR. The Clinical Impact of the C0/D Ratio and the CYP3A5 Genotype on Outcome in Tacrolimus Treated Kidney Transplant Recipients. Front Pharmacol. 2020 Jul 31;11:1142.

70. Koyama H, Cecka JM, Terasaki PI. Kidney transplants in black recipients. HLA matching and other factors affecting long-term graft survival. Transplantation. 1994 Apr 15;57(7):1064-8. PMID: 8165704.

71. Thervet E, Anglicheau D, King B, Schlageter MH, Cassinat B, Beaune P, et al. Impact of cytochrome P450 3A5 genetic polymorphism on tacrolimus doses and concentration-to-dose ratio in renal transplant recipients1 2. Transplantation. 2003 Oct 27;76(8):1233–5.

72. Narayanan M, Pankewycz O, El-Ghoroury M, Shihab F, Wiland A, McCague K, et al. Outcomes in African American Kidney Transplant Patients Receiving Tacrolimus and Mycophenolic Acid Immunosuppression. Transplantation. 2013 Feb 27;95(4):566–72.

73. Chamoun B, Torres IB, Gabaldón A, Sellarés J, Perelló M, Castellá E, et al. Progression of Interstitial Fibrosis and Tubular Atrophy in Low Immunological Risk Renal Transplants Monitored by Sequential Surveillance Biopsies: The Influence of TAC Exposure and Metabolism. JCM. 2021 Jan 4;10(1):141.

74. André C, Choukroun G, Bennis Y, Kamel S, Lemaire-Hurtel AS, Masmoudi K, et al. Potential interactions between uraemic toxins and drugs: an application in kidney transplant recipients treated with calcineurin inhibitors. Nephrology Dialysis Transplantation. 2021 Mar 30;gfab114.

75. Collins JM, Nworu AC, Mohammad SJ, Li L, Li C, Li C, et al. Regulatory variants in a novel distal enhancer regulate the expression of CYP3A4 and CYP3A5. Clinical Translational Sci. 2022 Sep 20;cts.13398.

76. Tornatore KM, Meaney CJ, Attwood K, Brazeau DA, Wilding GE, Consiglio JD, et al. Race and sex associations with tacrolimus pharmacokinetics in stable kidney transplant recipients. Pharmacotherapy. 2022 Feb;42(2):94–105.

77. Ferraris JR, Argibay PF, Costa L, Jimenez G, Coccia PA, Ghezzi LFR, et al. Influence of CYP3A5 polymorphism on tacrolimus maintenance doses and serum levels after renal transplantation: Age dependency and pharmacological interaction with steroids: CYP3A5 genotype on TAC and steroids. Pediatric Transplantation. 2011 Aug;15(5):525–32.

78. Gijsen V, Mital S, van Schaik RH, Soldin OP, Soldin SJ, van der Heiden IP, et al. Age and CYP3A5 genotype affect tacrolimus dosing requirements after transplant in pediatric heart recipients. The Journal of Heart and Lung Transplantation. 2011 Dec;30(12):1352–9.

79. Passey C, Birnbaum AK, Brundage RC, Oetting WS, Israni AK, Jacobson PA. Dosing equation for tacrolimus using genetic variants and clinical factors: Tacrolimus dosing equation. British Journal of Clinical Pharmacology. 2011 Dec;72(6):948–57.

80. Stratta P, Quaglia M, Cena T, Antoniotti R, Fenoglio R, Menegotto A, et al. The interactions of age, sex, body mass index, genetics, and steroid weight-based doses on tacrolimus dosing requirement after adult kidney transplantation. Eur J Clin Pharmacol. 2012 May;68(5):671–80.

81. Andrews LM, de Winter BCM, Tang JT, Shuker N, Bouamar R, van Schaik RHN, et al. Overweight Kidney Transplant Recipients Are at Risk of Being Overdosed Following Standard Bodyweight-Based Tacrolimus Starting Dose. Transplantation Direct. 2017 Feb;3(2):e129.

APPENDICES

Appendix 1: Data Abstraction Form

SECTION A: Demographic Data:

A1: Date of kidney transplant (dd/mm/yyyy): __ /___ /_____

A2: Age of recipient at transplant (years): _____

A3: Recipient sex: (1) Female (2) Male

A4: Weight of recipient (kilograms): _____

A5: Height of recipient (metres): _____

A6: Donor sex: (1) Female (2) Male

A7: Weight of donor (kilograms): _____

A8: Height of donor (metres): _____

A9: Donor age at nephrectomy (years): _____

A10: Donor creatinine from DTPA scan: _____

A11: Recipient co-morbidities / aetiology of CKD:

I. Diabetes: (1) Yes (2) No

II. Hypertension: (1) Yes (2) No

III. HIV: (1) Yes (2) No

IV. Glomerulonephritis. (1) Yes (2) No

V. Polycystic Kidney Disease: (1) Yes (2) No

VI. Obstructive uropathy: (1) Yes (2) No

VII. SLE: (1) Yes (2) No

VIII. Others: (1) Yes (2) No

A12: Dialysis vintage time (months): _____

A13: HLA mismatch: _____

A14: Induction therapy: (1) Yes (2) No

A15: Delayed allograft function: (1) Yes (2) No

SECTION B: Medication-related variables:

	At hospital Discharge	1st (± 1 wk.) month	3rd (± 3wks) month	6th (± 1) month	12th (± 1) month
B1: Haematocrit					
B2: Albumin					
B3: Tacrolimus trough levels (ng/ml)					
B4: Tac total daily dose (mg)					
B5: Antiproliferative agent i. None (0) ii. MPA (1) iii. AZA (2)					
B6: Creatinine					
B7: Steroid dose					

SECTION C: Outcome variables:

C1. Creatinine at one year (µmol/L): _____

C2: Number of clinical rejection episodes: _____

C3: Number of biopsy-proven rejection: _____

C4: Post-transplant diabetes mellitus: (1) Yes (2) No

If yes, indicate date of diagnosis (dd/mm/yyyy): __/__/____

C5: Post-transplant Infectious complications:

i. CMV: (1) Yes (2) No

If yes, indicate date of diagnosis (dd/mm/yyyy): __/__/____

ii. Disseminated fungal infections: (1) Yes (2) No

If yes, indicate date of diagnosis (dd/mm/yyyy): __/__/____

iii. Tuberculosis (TB): (1) Yes (2) No

If yes, indicate date of diagnosis (dd/mm/yyyy): __/__/____

iv. BK polyoma virus (1) Yes (2) No

If yes, indicate date of diagnosis (dd/mm/yyyy): __/__/____

C6: Return to dialysis within one year: (1) Yes (2) No

If yes, indicate date of diagnosis (dd/mm/yyyy): __/__/____

Appendix 2: KNH/UoN Ethics Review Committee Approval Letter

UNIVERSITY OF NAIROBI
FACULTY OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/244

28th June, 2022

Dr. Davies Otieno
Reg. No.H58/37708/2020
Dept. of Clinical Medicine & Therapeutics
Faculty of Health Sciences
University of Nairobi

Dear Dr. Otieno,

RESEARCH PROPOSAL: PATTERNS OF TACROLIMUS METABOLISM AND KIDNEY ALLOGRAFT OUTCOMES AT THE KENYATTA NATIONAL HOSPITAL (P271/03/2022)

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P271/03/2022.** The approval period is 28th June 2022 – 27th June 2023.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by KNH-UoN ERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to KNH-UoN ERC 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to KNH-UoN ERC.

PLAGIARISM REPORT:

PATTERNS OF TACROLIMUS METABOLISM AND KIDNEY ALLOGRAFT OUTCOMES AT THE KENYATTA NATIONAL HOSPITAL

ORIGINALITY REPORT

9%	5%	7%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	D. O, J. Kayima, S. McLigeyo, S. Ilovi, J. Ngigi, F.C.F. Otieno, E. Omonge, E.A. Onyango, S. Kabinga. "WCN23-0865 TACROLIMUS BLOOD TROUGH TO DOSE RATIO AS A SURROGATE TO TACROLIMUS METABOLIC PHENOTYPES IN KIDNEY TRANSPLANT RECIPIENTS: A SINGLE CENTRE ANALYSIS IN KENYA", Kidney International Reports, 2023 Publication	1%
2	erepository.uonbi.ac.ke:8080 Internet Source	1%
3	"Oral Abstracts", American Journal of Transplantation, 2017 Publication	<1%

LEAD SUPERVISOR AND CHAIRMAN'S APPROVAL:

This dissertation has been submitted with the approval of my lead supervisor and the chairman of the Department of Clinical Medicine and Therapeutics, University of Nairobi.

Lead Supervisor:

Dr. Syokau Ilovi

Lecturer

Consultant Physician and Medical Geneticist

Department of Clinical Medicine, and Therapeutics

Signed: ..... **Date:** 7.11.2023

Chairman of the department:

Prof. E. O. Amayo

Chairman

Consultant Physician and Neurologist

Department of Clinical Medicine, and Therapeutics

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF CLINICAL MEDICINE & THERAPEUTICS
P.O. Box 19676-00202 NAIROBI

Signed: ..... **Date:** 9/11/2022