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Whole-Blood Tacrolimus Trough Concentration in Renal Transplant Recipients at a Tertiary Care Center in Central Nepal: A Cross-sectional Study

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ABSTRACT

Introduction

Tacrolimus, a widely used immunosuppressant for renal transplantation, requires careful monitoring due to its narrow therapeutic index and high pharmacokinetic variability. Hence, this study aimed to evaluate the whole blood tacrolimus trough levels in post-renal transplant patients at TUTH.

Methods

A total of 257 patients who had undergone kidney transplantation were included in this descriptive cross-sectional study. Whole-blood tacrolimus concentration was measured using the ARCHITECT i1000SR analyzer (Abbott Diagnostics, North Chicago, USA) by CMIA. Ethical approval was taken from the Institutional Review Committee of IOM [Ref. No.: 448(6-11) E^2 081/082].

Results

Among the 257 renal transplant recipients, 197 (76.65%) were male and 60 (23.35%) were female, with a mean age of 38.06±10.74 years. The mean tacrolimus trough level was 7.58±3.92 ng/mL, with a median of 6.8 (IQR: 5.4- 9.1) ng/ml. Females had a slightly higher median tacrolimus concentration [7.2 (IQR: 5.7- 9.1)] compared to males [6.8 (IQR: 5.7- 9.1)]. Of the determinations, 175 (68.09%) were within the therapeutic range (5-15 ng/mL), 68 (26.46%) were below it, and 14 (5.45%) had elevated tacrolimus levels.

Conclusion

The median tacrolimus trough level in this study was 6.8 ng/mL (IQR: 5.4–9.1), slightly higher but within the therapeutic range compared to similar studies done in similar settings. Monitoring of tacrolimus trough concentrations is of utmost importance in the management of kidney transplant recipients.

Keywords

Chemiluminescent microparticle immunoassay; kidney transplantation; tacrolimus; therapeutic drug monitoring

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INTRODUCTION

Tacrolimus (FK-506) is an immunosuppressant macrolide that inhibits T- cell activation by binding to the FK- binding protein (FKBP).[1] It effectively prevents graft-versus-host disease (GVHD) and is the most widely used calcineurin inhibitor in Nepal for renal transplantation.[2] However, it requires careful therapeutic monitoring due to its narrow therapeutic index as incorrect dosing can lead to graft rejection or nephrotoxicity and neurotoxicity.[3] Globally, the use of Tacrolimus has been the mainstay of immunosuppressive regimens reported in >90% of kidney transplant recipients.[4]

In 2019, the Immunosuppressive Drugs Scientific Committee of the International Association of Therapeutic Drug Monitoring and Clinical Toxicity (IATDMCT) released an updated consensus report on TDM for tacrolimus, covering advancements in pharmacokinetics, pharmacogenetics, pharmacodynamics, and immunologic biomarkers, to assist health care professionals in handling this immunosuppressive drug in solid organ transplantation.[5] Since immunosuppressive drugs like tacrolimus is used on a long-term basis, whole blood or plasma concentration measurement of tacrolimus in organ transplant patients could help to ensure compliance with the drug treatment protocol.[6]

This study aimed to evaluate the whole blood tacrolimus concentration in post-renal transplant patients in TUTH.

METHODS

This is a descriptive cross- sectional study conducted from November 1, 2023 to May 31, 2024, in the Department of Clinical Biochemistry of TUTH. Ethical approval for this study was obtained from the Institutional Review Committee of Institute of Medicine under Reference Number 448(6-11)E[2] 081/082. All research procedures and methodologies were conducted in strict adherence to the relevant ethical guidelines and regulations to ensure the integrity and ethical soundness of the study. Before being released for analysis, the clinical data were anonymized and de-identified to ensure patients' confidentiality. The convenience sampling technique was used.

Based on a study done by Rodríguez-Perálvarez et al.[7], the standard deviation of trough tacrolimus level was 1.1.

Hence, sample size calculation was done using the following formula:

$$n = [z_\alpha \sigma/E]^2$$

where,

$z_\alpha = 1.65$

$\sigma = 1.1$

$E = 10\%$[8]

Hence, $n = [1.65 \times 1.1/0.1]^2$

$n = [1.815/0.1]^2$

$n = 329.42$

For the finite population, the sample size formula is adjusted. The adjusted formula is:

$$n_{adj} = n/[1+\{(n-1)/N\}]$$

where,

n_{adj} = Adjusted sample size for a finite population

n = Sample size calculated using the formula for an infinite population

N = Finite population size (N=440)

$n_{adj} = 329.42/[1+\{(329.42-1)/440\}]$

$n_{adj} = 188.67$

The minimum sample size calculated was 188.67. We enrolled 257 renal transplant patient receiving tacrolimus in this study.

A total of 257 EDTA blood samples from kidney transplant patients at TUTH (Kathmandu, Nepal) who were under tacrolimus treatment, were enrolled in the study. To maintain consistency in sample collection and minimize variability in drug concentration measurements, all blood samples were drawn in the morning, just before the administration of the next scheduled dose of tacrolimus. This pre-dose or trough-level sampling approach was followed to accurately assess the lowest concentration of the drug in the bloodstream, which is critical for therapeutic monitoring and dose adjustments. EDTA blood samples from all the renal transplant patient under tacrolimus therapy irrespective of the dosage and their duration of treatment were included in our study while hemolyzed sample, lipemic sample, sample with insufficient volume, and samples other than EDTA was excluded from the study.

Tacrolimus levels in whole blood are tested routinely in TUTH by an ARCHITECT i1000SR analyzer (Abbott Diagnostics, North Chicago, IL, USA). This method employs a Chemiluminescent Microparticle Immunoassay (CMIA) where a pre- treated sample, an assay diluent, and anti- tacrolimus- coated microparticles are mixed. Tacrolimus in the sample binds to these microparticles. After a delay, an acridinium- labeled tacrolimus conjugate is added that competes for binding sites. The resulting chemiluminescent reaction is measured in relative light units (RLUs) after adding Pre-Trigger and Trigger Solutions. There is an inverse relationship between the tacrolimus concentration and RLUs detected by the ARCHITECT iSystem Optics. The limit of detection was ≤ 1.5ng/ml. Based on the recommendations of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) on the therapeutic drug monitoring of

tacrolimus the therapeutic range of tacrolimus was 5-15 ng/ml.[5]

After entering the data in Microsoft Excel 2016, statistical analysis was performed using IBM SPSS Statistics version 22.0. The continuous variables were expressed as mean ± standard deviation (SD) or median (IQR). The categorical variables were expressed as frequency and percentage.

RESULTS

A total of 257 samples received for tacrolimus assay were included in the study. Among the 257 renal transplant recipients recruited, 197 (76.65 %) were male and 60 (23.35 %) were female . The mean age of participants was 38.06 ± 10.74 years. The mean ± SD of tacrolimus levels measured using the ARCHITECT system are presented in, (Table 1). The median (IQR) of tacrolimus was 6.8(IQR: 4.9-9.2). The tacrolimus concentrations ranged from a minimum of 0.50 ng/mL to a maximum of 25.70 ng/ml.

The tacrolimus trough concentration was higher in female renal transplant patients, with a median (IQR) of [7.2 (IQR: 5.7- 9.1)], compared to males, who had a median of 6.8(IQR: 5.4- 9.1) ,(Table 2). Despite the marginally higher median concentration observed in females, the maximum tacrolimus level recorded was notably higher in males, measuring 39.50 ng/mL, while in females it reached 23.40 ng/ml.

Of the total 257 tacrolimus determinations analyzed in this study, the majority, 175 (68.09 %) were within the therapeutic range (5-15 ng/mL), 68(26.46 %) were below this range, and 14 (5.45 %) were above the range, (Figure 1). The median age of patients with tacrolimus levels above 15 ng/mL was higher compared to those within the therapeutic range

Table 1. Descriptive statistics of anthropometric and biochemical parameters in renal transplant recipients (n= 257)

Characteristics	Mean ± SD
Age (years)	38.06 ± 10.74
ARCHITECT Tacrolimus (ng/mL)	7.58 ± 3.92

Table 2. Differences in the Tacrolimus level based on sex

ARCHITECT Tacrolimus	Male (n=197)	Female (n=60)
Median (IQR)	6.8 (5.4,9.1)	7.2 (5.7,9.1)
Minimum	1.32	2.16
Maximum	39.50	23.40

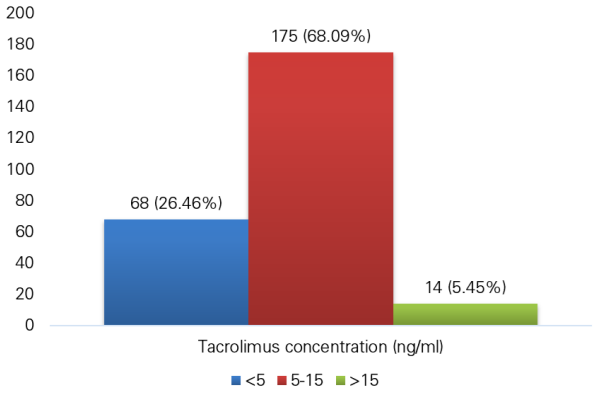

Figure 1. Bar diagram showing the whole- blood trough tacrolimus concentrations

and below it, at 43(IQR: 35.75- 47.0) years versus 36(IQR: 30.0- 43.0) years and 38(IQR: 32.0- 46.75) years respectively. Patients with tacrolimus levels within the therapeutic range had the lowest median age of 36 (IQR: 30.0- 43.0) years.

DISCUSSION

Tacrolimus is an immunosuppressant macrolide which is useful for the prophylaxis of graft- vs- host disease (GVHD) when used alone, or in conjunction with other immunosuppressive drugs.[9] Therapeutic monitoring of Tacrolimus is an integral part of optimizing drug therapy in transplant patients because of its narrow therapeutic index and high variability of pharmacokinetics among individuals.[3] Since immunosuppressive drugs are used on a long-term basis, measurement of whole blood or plasma concentration of immunosuppressive drugs in organ transplant patients will help in assuring compliance with the drug treatment protocol.[10]

In this study, the mean tacrolimus trough level in renal transplant recipients was found to be 7.58 ± 3.92 ng/ml, with a median of 6.8(IQR: 5.4- 9.1) ng/ml. Similar results were reported by Goodall et al., who observed an overall mean trough tacrolimus level of 6.95 ± 1.53 ng/ml which is lower than that of the value observed in our study. [11] The mean age of the enrolled renal transplant patients was 38.06 ± 10.74 years, with a higher prevalence of males (76.65 %) compared to females (23.35 %). These findings align with those of Shrestha et al., who reported a mean age of 33.7 ± 10.83 years, with 70.2 % male and 29.8 % female participants.[12]

The increased prevalence of renal transplantation among individuals under 40 years old can be attributed to chronic glomerulonephritis, the most common cause of end stage renal disease (ESRD) in younger population.[13] While several studies have indicated that women tend to experience a higher prevalence of chronic kidney disease (CKD) compared to men, with factors such as hormonal

influences, differences in renal physiology, and variations in healthcare-seeking behavior, the progression to end- stage renal disease appears to be more pronounced in men, as research have suggested the incidence of ESRD being approximately 1.5 times greater in men than in women.[14] Data suggest that women are less likely to receive deceased or living donor kidney transplants but are more frequently living kidney donors.[15,16] The etiology of CKD also varies by sex; diabetes and hypertension are more prevalent causes in men, whereas autoimmune diseases, such as systemic lupus erythematosus, are more common among women.[17] Although biological factors contribute to a more rapid progression of CKD in men, gender-based social and cultural differences often result in delayed initiation or lack of access to kidney replacement therapy for women, including later referrals for transplant evaluation.[16]

The median tacrolimus trough level was found to be higher in females than in males. Research on kidney transplant outcomes by sex has yielded inconclusive and often conflicting results. While some studies have reported worse outcomes for women, others find similar outcomes between the sexes or suggest that male sex is an independent risk factor for poorer outcomes. [18-20] The biological sex of kidney transplant recipients influences the pharmacokinetics and pharmacodynamics of immunosuppressive drugs like tacrolimus, which exhibits high intra-patient variability. Studies have indicated that women have higher weight - normalized clearance of tacrolimus,[21] although explanations for this finding remain inconclusive, and sex- specific dosage recommendations are not yet implemented.[22] Furthermore, Laprise et al. highlight that women may experience higher rates of graft injury due to factors such as pregnancy and stronger immune responses.[23]

In our study, most of the patients (68.9 %) had tacrolimus levels within the therapeutic range (5- 15 ng/mL), while 26.46 % were below this range and 5.45 % had elevated levels of tacrolimus. In contrast to our study, research by Robles Piedras reported only 48.4 % within the therapeutic range, with 7.1 % below and 44.5 % above it.[24] This discrepancy may be attributed to the lower therapeutic range used in their hospital (4-14 ng/mL). While some authors recommend a therapeutic range of 10 to 20 ng/mL during the early post- transplant period, concentrations above 20 ng/mL can increase the risk of adverse events. Patients with tacrolimus levels exceeding 15 ng/mL were older than those within the therapeutic range. This trend may be attributed to the need for higher doses of immunosuppressants in older populations, which is associated with an increased risk of elevated drug levels. [25]

Patients with low tacrolimus level may be at risk of underexposure and alloimmune-mediated injury according to immunological risk while those with higher level may be prone to overexposure and toxicity. Tailored immunosuppressive strategy through trough tacrolimus measurement can be beneficial in finding the lowest effective dose of immunosuppressive medication to control the alloimmune response while minimizing drug toxicity.[26]

However, our study is confined to analyzing whole blood tacrolimus status in the renal transplant group. We were not able to collect data on tacrolimus dosages or associated complications. Nevertheless, this study is the first to describe tacrolimus concentrations measured at TUTH, which could aid in the management of renal transplant patients and help mitigate adverse outcomes.

CONCLUSION

The mean tacrolimus trough level in renal transplant recipients in the current study was found to be 7.58±3.92 ng/ml, with a median of 6.8 (IQR: 5.4-9.1) ng/ml, which was slightly higher yet within therapeutic range than that observed in other similar studies done in similar settings. Monitoring and maintaining of stable tacrolimus trough concentrations is of utmost importance in the management of kidney transplant recipients. Whole blood trough tacrolimus measurement is an essential service for maintaining the tacrolimus concentration within therapeutic range which helps in management of renal transplant patients and ease their post-transplant journey.

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CONFLICT OF INTEREST

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