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Role of pharmacodynamic markers in monitoring tacrolimus activity in renal transplant patients: A systematic review.

Pingali Usha Rani[1*], Mekala Padmaja[1], Gangadhar Taduri[2], Mohammed Abid Ali[1], Imran Khan[1]

[1]Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India

[2]Department of Nephrology, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India

Abstract

Background: Tacrolimus is an immunosuppressive agent that is prescribed to prevent acute rejection following solid organ transplantation such as kidney, liver, and heart transplantations. It is widely used agent, however, characterized by narrow therapeutic index and high inter-individual variability in dose requirement necessitating frequent therapeutic drug monitoring to prevent acute rejection or renal toxicity. The primary mechanism by which Calcineurin Inhibitors (CNIs) depress the immune system is by impairing the T helper cell's calcineurin-Nuclear Factor of Activated T cells (NFAT) signaling pathway. Individual differences exist in the drug sensitivity and CNI distribution into T cells. Because of this, measuring pharmacodynamic indicators linked to the suppression of calcineurin may better represent the biological action of CNIs in specific individuals. Systemic CNI trough concentrations are not always a good predictor of the biological activity of the medications in immune cells.

Aim: To conduct a comprehensive review on the use of pharmacodynamic indicators (calcineurin phosphatase activity and nuclear factor of activated T-cells activity) in renal transplant patients' monitoring of tacrolimus activity.

Methods: A systematic search was conducted in PubMed, Cochrane databases to collect and analyze all studies published from year 1995 till 2021; that investigated the role of Pharmacodynamic markers (Calcineurin phosphatase activity and Nuclear Factor of Activated T-cells activity) in monitoring tacrolimus activity in renal transplant patients. Randomised controlled trials and observational studies were included.

Results: Two studies met the inclusion criteria. The clinical value of pharmacodynamic monitoring on tacrolimus based renal transplant studies based on NFAT related gene expression should be evaluated in more prospective studies.

Conclusion: Our review indicates that the measurement of pharmacodynamic biomarkers plays an important role in monitoring tacrolimus activity in renal transplant patients.

Keywords: Transplantation, Tacrolimus, Pharmacodynamic markers, Calcineurin phosphatase, NFAT.
Abbreviations: Tac: Tacrolimus; NFAT: Nuclear Factor of Activated T-Cell; CN: Calcineurin; CNI: Calcineurin Inhibitors; PD: Pharmacodynamics; PK: Pharmacokinetics; Tx: Transplantation; DSA: Donor Specific anti-HLA Antibodies; PBMC: Peripheral Blood Mononuclear Cell; CYP3A: Cytochrome P450 3A; MeSH: Medical Subject Headings; MRE: Mean Residual Expression; RGE: Residual Gene Expression.

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Introduction

Renal transplant often involves patients with End-Stage Kidney Disease (ESRD) [1]. Kidney transplantation can be cadaveric (deceased-donor) or living-donor. Living-donor kidney transplants are further categorized as genetically related (living-related) or non-related (living-unrelated) transplants, depending on whether a biological relationship exists between the donor and recipient [2]. Kidney transplant recipients must remain on immunosuppressant's for the rest of their life to prevent their body from rejecting the new kidney [3]. Tacrolimus is cornerstone of immunosuppressive therapy [4] and has become the calcineurin inhibitor of choice for prevention of

acute rejection following solid organ transplantation such as kidney, liver, and heart [5]. The primary mechanism by which Calcineurin Inhibitors (CNIs) reduce immune response is by impairing the T helper cell Nuclear Factor of Activated T cells (NFAT) signaling pathway. The liver and proximal small intestine where Cytochrome P450 3A (CYP3A) enzymes break down tacrolimus. Tacrolimus only has a 25% oral bioavailability when taken orally due to substantial pre-systemic metabolism in the GIT [6].

Tacrolimus has a narrow therapeutic index, necessitating routine therapeutic drug monitoring in order to keep concentrations within the therapeutic range and avoid acute rejection or renal damage [7]. Monitoring of Tacrolimus concentrations in whole blood is used to establish correlations between drug concentration and its effect [8]. Sub therapeutic concentrations in early post-transplant period increase the risk of rejection while concentrations above the target range contribute to drug-related toxicity [9]. Acute cellular rejection can occur when tacrolimus concentration is within target concentration range, demonstrating that tacrolimus whole blood concentrations do not always fully reflect its pharmacological effect. Tacrolimus is dosed according to therapeutic drug monitoring schemes based on pharmacokinetics, which are very variable. Elucidation of the mechanism of immunosuppressive action has led to the hope that dosing according to effect of the drugs rather than according to the amount in the blood at any given time, might decrease toxicity and increase graft survival. Such a pharmacodynamic approach would involve determining calcineurin phosphatase activity instead of blood levels of the given drug [10]. Pharmacodynamic monitoring is proposed as a new strategy to provide information about the biological effect and the degree of immunosuppression. It has been recognized as a helpful tool to evaluate efficacy and to optimize drug dosing [11]. Pharmacodynamic (PD) biomarkers for Tacrolimus monitoring are drug specific and drug non-specific. Drug specific PD biomarkers are CN activity and NFAT-regulated gene expression. Drug non-specific PD biomarkers are Intracellular cytokines (Interleukin-2, Interferon-γ), Donor Specific anti- HLA Antibodies (DSA), donor derived cell free DNA (dd cf DNA). In the case of tacrolimus, drug- specific biomarkers are related to the signal transduction pathways and enzyme activities inhibited by the drug, whereas nonspecific biomarkers reflect the inhibition of T-cell activation and proliferation in general, including cytokine production. PD biomarkers can be estimated either directly in whole blood, whole blood stimulated with mitogens, antibodies, in donor leukocytes or in isolated lymphocytes12,13. Calcineurin activity measurement has been proved as a Tacrolimus-specific PD parameter. Calcineurin activity assays directly measure the effect of tacrolimus on its target enzyme CN. Calcineurin is a serine–threonine-specific, Ca ++ -calmodulin-activated protein phosphatase.

There are four known phosphatases in the blood that could liberate phosphate from the peptide (PP1, PP2A, PP2C and PP2B=calcineurin) [12]. The transcription factor family known as NFAT has been shown to be particularly susceptible to suppression by the drug tacrolimus. The identification of kidney transplant patients who are more likely to have acute rejection, opportunistic infections, malignancy, and cardiovascular risk is supported by the determination of residual NFAT-regulated gene expression. Maguire *et al*., showed an inverse correlation between tacrolimus concentrations and nuclear translocation of NFAT1 in renal transplant recipients [13]. Two other studies showed a correlation between lower NFAT-regulated gene expression and increased frequency of infection episodes in transplant patients [14,15]. Translocation of NFAT1 to Peripheral Blood Mononuclear Cell (PBMC) nuclei is a suitable candidate biomarker to monitor Tacrolimus PD after transplantation [16]. Monitoring of NFAT - regulated gene expression complements tacrolimus PK in guiding the therapy.

Rationale

A high intra-patient variability with Tacrolimus is considered a risk factor for poor long-term outcomes after transplantation. Recent reports indicate that an understanding of within patient variability of Tacrolimus concentrations could be a useful tool for optimizing the immunosuppressive therapy in solid organ transplantation. In the literature, CN activity, NFAT have been reported as drug specific PD biomarkers. As Tacrolimus is a narrow therapeutic index drug, small variations in concentrations can lead to large differences in PD response influencing the graft and overall clinical outcomes. Pharmacodynamic biomarkers could provide more prognostic and diagnostic information regarding the risk of rejection and condition of allograft at an earlier time point and allow anti-rejection therapy to be adjusted before serious injury ensues. This systematic review is intended to generate evidence for the role of pharmacodynamic markers- CN and NFAT activity in renal transplant patients on tacrolimus regimen from the existing literature.

Materials and Methods

The protocol is registered with PROSPERO (CRD42021281547). The study was reported as per Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA-2020) guidelines.

Literature search strategy and document retrieval

A comprehensive and systematic search of the published literature for trials of dose adjustment of tacrolimus in patients who underwent renal transplantation using Calcineurin (CN) phosphatase activity or NFAT-regulated gene expression was conducted from PubMed, Cochrane databases from literature published between 1995 to 2021. A systematic search was conducted using the phrase: “Pharmacodynamic markers in monitoring tacrolimus activity in renal transplant patients”. The reference list of

each relevant publication was also examined to identify additional studies.

Eligibility criteria

Selection criteria were based on tacrolimus concentrations, duration of therapy; outcomes of the studies, randomized controlled trials were included within analysis. Non-randomized trials, clinical observations, animal studies and studies in non-English languages were excluded.

• Only articles in English language were included.

• The data after year 1995 was selected for inclusion in our study.

• The data included tacrolimus trough concentration on pre-transplantation and post-transplantation.

• The data included tacrolimus dose on pre-transplantation and post-transplantation.

Of these, 02 duplicates were removed. The inclusion criteria were original research articles. Another 43 results were further excluded by screening the title and abstract. Finally, selected studies were 02, Data was extracted from these 02 studies for Systemic Review.

Selection criteria

Published Randomized Control Trials (RCT) and Observational studies on renal transplant patients who were on tacrolimus regimen where pharmacodynamic markers (Calcineurin phosphatase activity and Nuclear Factor of Activated T-cells activity) were analyzed.

Data extraction

For this review, information was extracted including primary author, country of origin, publication time, number of subjects, Gender, control intervention, duration of treatment intervention, dose and duration of intervention, outcomes, and clinical efficacy. A combination of Medical Subject Headings (MeSH) terms and free text was used, combined with Boolean logical operators to construct the search strategy. We did not use any restrictions in the electronic search for trials except for a language restriction (non-English languages were excluded) (Figure 1).

Results and Discussion

Two studies meeting the eligibility criteria were included for the analysis. Figure 1 shows the inclusion of studies. A randomised controlled trial by Alison B. Webber *et al*., [17], 40 stable kidney transplant recipients received tacrolimus over 1 year, dose adjustments for tacrolimus were done based on the Mean Residual Expression in the intervention arm compared to dose adjustment based on trough levels of tacrolimus in the control arm. The other study, by Sara Bremer *et al*., [18], an observational study was done to characterize Nuclear Factor of Activated T cells (NFAT)-regulated gene expression as a potential pharmacodynamic biomarker in 29 renal transplant patients to individualize tacrolimus therapy. The study characteristics are provided in the Tables 1-3.

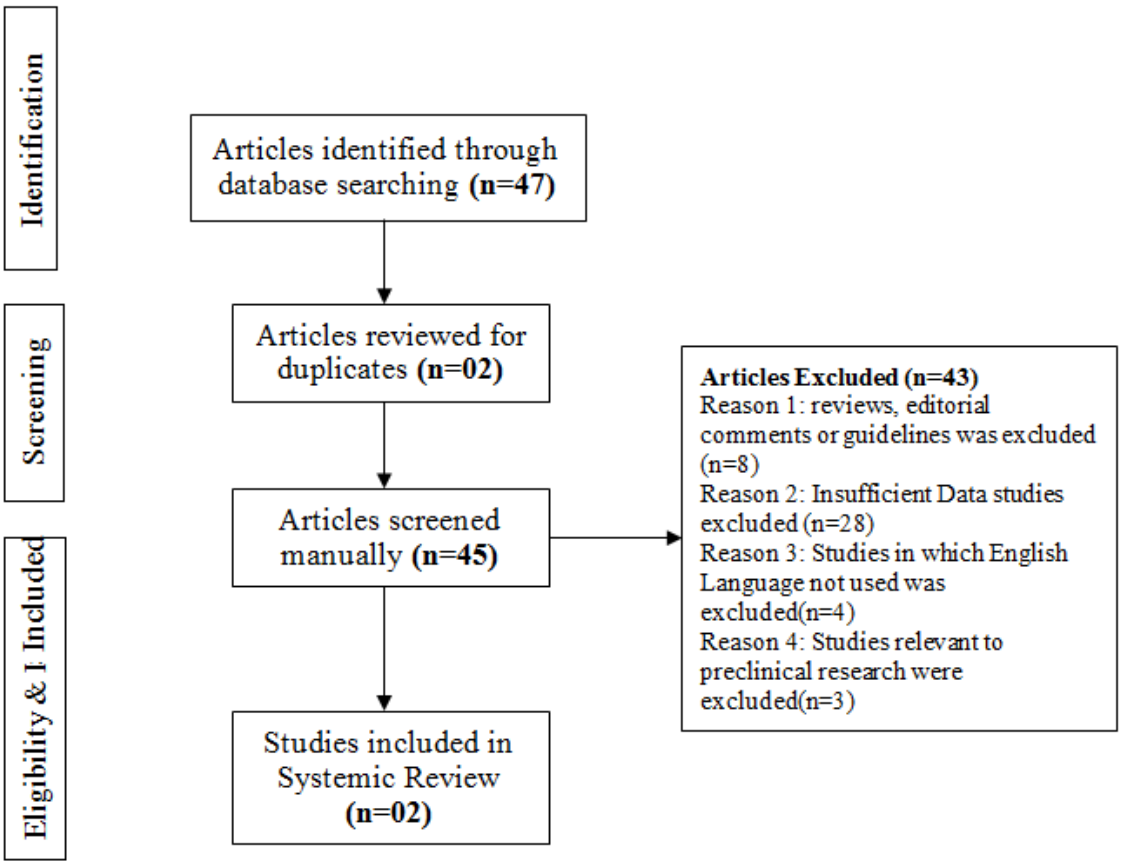

Figure 1. *Flow diagram showing preferred reporting items for systematic review.*

Table 1. *Baseline characteristics of study groups.*

First Author Name	Year	Country	Sample size (n)	Gender(M/F)*
Allison B. Webber	2018	USA	40	24/16
Sara Bremer	2017	Norway	30	24/5
Note: *M: Male, F: Female				

Table 2. *Comorbid conditions in study groups.*

Author	Diabetics		Glomerulonephritis		Hypertension		Others	
	Intervention	Control	Intervention	Control	Intervention	Control	Intervention	Control
Allison B. Webber	1	3	11	9	5	6	3	2
Sara Bremer	3		4		_		22	

Table 3. *Clinical characteristics of study groups.*

Author	Tac Trough Pre-Tx/Post-Tx (µg/L)		Tac Dose Pre-Tx/Post-Tx		No. of subjects with infections		Acute Graft Rejection	% MRE and RGE	
	Intervention	Control	Intervention	Control	Intervention	Control		Intervention	Control
Allison B. Webber	9.9/7.9	8.8/6.6	2000/1750 (mg/d)	2000/1500 (mg/d)	10	6	6	41.4 (25.2-61.6)	25.3 (20.3-37.2)
Sara Bremer	5.2/5.4		0.060/0.048 (mg kg–1 day–1)		3		2	40.6 (39.7–41.6)	

Tacrolimus is one of the important immunosuppressants for the prevention of acute rejection and has been the cornerstone of immunosuppressive regimens in renal transplant. The prolonged-release formulation may improve compliance and possibly long-term outcomes. Tacrolimus has significantly improved the outcomes of renal transplant, including reduction of acute rejection, improvement of renal function, graft survival, and reduction of some of the adverse effects associated with Cyclosporine (CsA). Tacrolimus based on NFAT-dependent cytokine appears to be more feasible without any signs and symptoms in infectious complications, malignancies or rejections based on tacrolimus trough levels.

In the study by Alison B. Webber, the patients whose tacrolimus dose was not adjusted in the 6 months, Kidney Transplant Recipient (KTR) with infections had statistically lower MRE compared with those without infections. The differences at 1 year trended toward lower MRE for those with infections, but it did not reach statistical significance (P=0.20). This finding suggests that a lower MRE is also associated with infectious complications. A higher MRE did not reflect inadequate cytokine suppression putting patients at risk for rejection. This is in line with other studies using this assay that found a well-defined low MRE cutoff associated with infection and malignancy, with a less well defined and more variable range of MRE cutoff associated with rejection [19-21]. Increasing tacrolimus dose based on high MRE may have been responsible for more infectious complications.

There is possibility of utilizing NFAT-regulated gene expression measurements as a tool for improvement of monitoring Tacrolimus dose regimen. Studies have reported Residual Gene Expression (RGE) [22] as a promising pharmacodynamic biomarker of the CNI response. However, the bulk of investigations to date have been on CsA (Cyclosporin) treated long-term stable transplant patients. The study by Sara Bremer et.al investigated NFAT-regulated gene expression in 29 renal allograft recipients treated with Tacrolimus in the early phase after Transplantation. Patients' cytokine gene expression levels varied greatly before transplantation and 1.5 hours after Tacrolimus dosage (E1.5). The observed variation in baseline expression (E0) might be attributed to variations in the underlying illness, comorbidities, pharmacological therapy, genetics, immunological condition, and surgical response. The heterogeneity in baseline cytokine levels is significantly eliminated by normalisation of E1.5 to E0, expressed as RGE, which also enables more precise evaluation of the medication response following dosing. The observed time-dependent changes in gene expression response indicate that single gene expression measurements may not be sufficient to predict the pharmacodynamic response of Tacrolimus.

Conclusion

Tacrolimus dosage can be changed based on nuclear factor of activated T cell-regulated gene expression measurements. This method may be particularly useful in cases of severe cytokine inhibition since it provides a more accurate picture of immunosuppression than trough levels provide. The major drawbacks of tacrolimus-based

treatment are constant worries about toxicities and side effects brought on by inescapable immunosuppression, not a lack of effectiveness. There is a need for studies that have a large enough sample size to evaluate the safety of employing quantitative measurement of NFAT-regulated gene expression as a test to reduce tacrolimus dosage. Future research is necessary to determine the best MRE cut-off for reducing tacrolimus as well as to fully explore the potential of NFAT-dependent cytokine expression for pharmacodynamics monitoring. However, more information is required, considering various patient demographics, interfering variables, monitoring techniques, and goal ranges. Finally, prospective clinical trials should assess the clinical utility of pharmacodynamic monitoring based on NFAT-regulated gene expression.

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***Correspondence to:**

Usha Rani P

Department of Clinical Pharmacology and Therapeutics

Nizam's Institute of Medical Sciences

Hyderabad

Telangana

India