Clinical research article

Correlating Tacrolimus levels with dose formulation: Implications for toxicity and renal graft rejection in a cohort of kidney transplant recipients with steroid-free maintenance immunosuppression

Natalia Gaitán-Torres[1], Nicolás Lozano-Suárez[2], Andrea García-López[2]*, Andrea Gómez-Montero[2] & Fernando Girón-Luque[2]

[1] Department of Transplant Pharmacology, Colombiana de Trasplantes, Av. Carrera 30 # 47A – 47, Bogotá D. C., Colombia
[2] Department of Transplant Research, Colombiana de Trasplantes, Av. Carrera 30 # 47A – 47, Bogotá D. C., Colombia

***Corresponding author E-mail:** aegarcia@colombianadetrasplantes.com
(+57) (3005024618)

Authors' contribution: All persons who meet authorship criteria are listed as authors. All authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the manuscript's concept, design, analysis, writing, or revision.

Received: February 16, 2024
Corrected: August 15, 2024
Accepted: August 22, 2024

https://doi.org/10.15446/rcciquifa.v54n1.113036

SUMMARY

Correlating Tacrolimus Levels with Dose Formulation: Implications for Toxicity and Renal Graft Rejection in a cohort of kidney Transplant Recipients with Steroid-free maintenance immunosuppression

Introduction: Chronic kidney disease (CKD) affects 10% of the worldwide population, being renal transplantation the optimal treatment for advanced stages. Post-transplant care involves immunosuppressive therapy, primarily utilizing tacrolimus, to prevent graft-rejection and failure. This study analyzes the correlation between tacrolimus blood levels, dosing, toxicity, and graft-rejection among renal transplant recipients with steroid-free maintenance immunosuppression in Colombia. **Methodology**: We conducted a retrospective cohort on kidney transplant patients followed at Colombiana de Trasplantes from 07-2019 to 06-2022, including patients with tacrolimus as immunosuppressive therapy that completed at least one year of post-transplant follow-up. Data on tacrolimus levels and transplant outcomes were collected at four intervals post-transplant. Bivariate analysis and unadjusted logistic regression were used to analyze the correlation and association between tacrolimus blood levels and tacrolimus doses, rejection, and toxicity. **Results**: The study included 368 patients. A weak correlation was found between variability of levels and dose (rho=0.31, p <0.001) but was not significant between tacrolimus doses and categorized blood levels. Acute graft-rejection occurred in 15.5% of patients, while only 3.8% experienced toxicity. No significant differences in rejection rates were observed with tacrolimus blood levels. **Conclusions**: This study found no significant

association between tacrolimus levels and graft-rejection, toxicity or categorized tacrolimus blood levels within the first-year post-transplant. However, the importance of tacrolimus level monitoring is underscored by its variability, influenced by dosing, adherence, and pharmacogenetics. Further research with a larger sample size is needed to improve our understanding of tacrolimus pharmacokinetics and encourage accurate reporting of toxicity in the Colombian population.

Keywords: Tacrolimus; graft rejection; toxicity; kidney transplantation; clinical pharmacology.

RESUMEN

Correlación de los niveles de tacrolimus con la formulación de la dosis: implicaciones para la toxicidad y el rechazo del injerto renal en una cohorte de receptores de trasplante renal con inmunosupresión de mantenimiento sin esteroides

Introducción: La enfermedad renal crónica (ERC) afecta al 10% de la población mundial, siendo el trasplante renal el tratamiento óptimo para estadios avanzados. El cuidado postrasplante implica terapia inmunosupresora, principalmente utilizando tacrolimus, para prevenir el rechazo y el fracaso del injerto. Este estudio analiza la correlación entre los niveles sanguíneos de tacrolimus, la dosis, la toxicidad y el rechazo del injerto entre los receptores de trasplante renal con inmunosupresión de mantenimiento sin esteroides en Colombia. **Metodología:** Realizamos una cohorte retrospectiva de pacientes trasplantados renales seguidos en Colombiana de Trasplantes desde 07-2019 hasta 06-2022, incluyendo pacientes con tacrolimus como terapia inmunosupresora que completaron al menos un año de seguimiento postrasplante. Los datos sobre los niveles de tacrolimus y los resultados del trasplante se recogieron en cuatro intervalos posteriores al trasplante. Se utilizó un análisis bivariado y una regresión logística no ajustada para analizar la correlación y la asociación entre los niveles sanguíneos de tacrolimus y las dosis de tacrolimus, el rechazo y la toxicidad. **Resultados:** El estudio incluyó a 368 pacientes. Se encontró una correlación débil entre la variabilidad de los niveles y la dosis (rho = 0,31, $p < 0,001$), pero no fue significativa entre las dosis de tacrolimus y los niveles sanguíneos categorizados. El rechazo agudo del injerto se produjo en el 15,5% de los pacientes, mientras que solo el 3,8% experimentó toxicidad. No se observaron diferencias significativas en las tasas de rechazo con los niveles sanguíneos de tacrolimus. **Conclusiones:** Este estudio no encontró una asociación significativa entre los niveles de tacrolimus y el rechazo del injerto, la toxicidad o los niveles sanguíneos categorizados de tacrolimus durante el primer año posterior al trasplante. Sin embargo, la importancia de la monitorización de los niveles de tacrolimus se ve subrayada por su variabilidad, influenciada por la dosificación, la adherencia y la farmacogenética. Se necesitan más investigaciones con un tamaño de muestra mayor para mejorar nuestra comprensión de la farmacocinética del tacrolimus y fomentar la notificación precisa de la toxicidad en la población colombiana.

Palabras clave: Tacrolimus; rechazo del injerto; toxicidad; trasplante renal; farmacología clínica.

RESUMO

Correlação dos níveis de tacrolimus com a formulação da dose: implicações para a toxicidade e rejeição do enxerto renal em uma coorte de receptores de transplante renal com imunossupressão de manutenção sem esteroides

Introdução: A doença renal crônica (DRC) afeta 10% da população mundial, sendo o transplante renal o tratamento ideal para estágios avançados. O tratamento pós-transplante envolve terapia imunossupressora, utilizando principalmente tacrolimus, para prevenir a rejeição e a falha do enxerto. Este estudo analisa a

correlação entre os níveis sanguíneos de tacrolimus, dosagem, toxicidade e rejeição do enxerto entre receptores de transplante renal com imunossupressão de manutenção sem esteroides na Colômbia. **Metodologia:** Conduzimos uma coorte retrospectiva em pacientes transplantados renais acompanhados na Colombiana de Trasplantes de 07-2019 a 06-2022, incluindo pacientes com tacrolimus como terapia imunossupressora que completaram pelo menos um ano de acompanhamento pós-transplante. Dados sobre os níveis de tacrolimus e resultados do transplante foram coletados em quatro intervalos pós-transplante. Análise bivariada e regressão logística não ajustada foram usadas para analisar a correlação e associação entre os níveis sanguíneos de tacrolimus e doses de tacrolimus, rejeição e toxicidade. **Resultados:** O estudo incluiu 368 pacientes. Uma correlação fraca foi encontrada entre a variabilidade dos níveis e dose (rho=0,31, p <0,001), mas não foi significativa entre as doses de tacrolimus e os níveis sanguíneos categorizados. A rejeição aguda do enxerto ocorreu em 15,5% dos pacientes, enquanto apenas 3,8% apresentaram toxicidade. Nenhuma diferença significativa nas taxas de rejeição foi observada com os níveis sanguíneos de tacrolimus. **Conclusões:** Este estudo não encontrou associação significativa entre os níveis de tacrolimus e a rejeição do enxerto, toxicidade ou níveis sanguíneos categorizados de tacrolimus no primeiro ano pós-transplante. No entanto, a importância do monitoramento dos níveis de tacrolimus é ressaltada por sua variabilidade, influenciada pela dosagem, adesão e farmacogenética. Mais pesquisas com um tamanho de amostra maior são necessárias para melhorar nossa compreensão da farmacocinética do tacrolimus e encorajar relatórios precisos de toxicidade na população colombiana.

Palavras-chave: Tacrolimus; rejeição de enxerto; toxicidade; transplante renal; farmacologia clínica.

1. INTRODUCTION

Chronic kidney disease (CKD) affects 10% of the world's population [1, 2], and it is divided into five stages depending on the progression of the disease. In the severe loss of renal function or late-stage CKD, renal transplantation is considered the best treatment option, improving the quality of life and patient survival [3]. Once a patient has been transplanted, immunosuppressive therapy is a fundamental part of organ maintenance, which prevents renal graft rejection.

Within immunosuppressive therapy, tacrolimus is one of the most widely used immunosuppressants. It is usually used in combination therapy with steroids and a purine synthesis inhibitor, such as mycophenolate [4]. In Colombia for the year 2020, the high-cost account (in Spanish Cuenta de Alto Costo – CAC) reported that immunosuppressive therapy with a three-drug regimen was used in 59% of cases and a two-drug regimen in 35.5% of renal transplants. Moreover, tacrolimus was part of the three and two-drug regimen in 93.6% and 83.8% of cases, respectively [3]. This demonstrates the relevance of tacrolimus and its wide use in immunosuppressive therapy.

Tacrolimus is a prodrug belonging to the group of calcineurin inhibitors. Its mechanism of action is that once it binds to the FKBP family protein. This binding inhibits the early-stage activation of T cells, preventing the activation of T lymphocytes and their IL-2-dependent proliferation. Consequently, this interruption hinders the entire activation cascade of cells, including B cells [5]. The plasma concentration of tacrolimus varies due to different intra- and interindividual factors [5]. Recognized for its narrow therapeutic [6] maintaining tacrolimus levels within a specific range is crucial for effective post-renal transplant patient following [7]. Achieving this balance is a significant challenge in clinical practice, requiring precise dosing tailored to each patient

[7]. This precision is vital to mitigate the risk of renal rejection and toxicity, a concern heightened during the initial post-transplantation year when renal graft rejection is most prevalent [8].

This study aims to explore the correlation between tacrolimus levels and formulated dose, and association with toxicity, and renal graft rejection in renal transplant patients. As there is variability in tacrolimus levels and limited local evidence on the relevance of these levels to renal transplant outcomes, the results of this study could help clinicians make informed decisions, individualize patient treatments, and increase the knowledge of pharmacists on the drug's pharmacovigilance and optimization of its use. Overall, this study has the potential to improve the outcomes of renal transplant patients and advance our understanding of the optimal use of tacrolimus.

2. METHODOLOGY

2.1. Type of Study

An observational, analytical, retrospective cohort study that included patients who underwent kidney transplantation in Colombiana de Trasplantes between July 2019 and June 2022. All patients received initially a steroid-free maintenance immunosuppression therapy with double therapy that included tacrolimus, some patients required steroid addition in the following by individualized reasons. All the included patients completed at least one year of post-transplant follow-up. There were no exclusions. A convenience sampling was performed, including all patients who met the selection criteria in the period under evaluation.

2.2. Data collection and study variables

Data were systematically extracted from electronic medical records, encompassing comprehensive information such as laboratory results, sociodemographic details, formulation specifics, and biopsy records. The study conducted four distinct review points over the course of the year, during which tacrolimus levels, formulation adjustments, and instances of acute rejection were meticulously examined. These assessment intervals occurred at the first month, third month, sixth month, and twelfth month post-transplantation. At the end of the following period, an overall incidence of toxicity and acute rejection were obtained.

Acute rejection was defined as a pathological diagnosis based on the Banff Classification of a renal graft biopsy. Tacrolimus blood levels were also categorized as decreased (<5 ng /ml), optimal (5-8 ng /ml) and elevated (>8 ng /ml). Finally, to determine the incidence of toxicity, the terms "toxicity, tacrolimus toxicity, tacrolimus nephrotoxicity, tacrolimus nephrotoxicity, tacrolimus nephropathy, tremor, acne, hair loss, gastrointestinal symptoms, tacrolimus nephrotoxicity, tacrolimus nephropathy, tremor, acne, hair loss, gastrointestinal symptoms" were used, tacrolimus nephrotoxicity, tacrolimus dose adjustment, over-immunosuppression, elevated tacrolimus levels, neurotoxicity, headache and elevated creatinine" as key terms in first-year post-transplant consultations.

2.3. Statistical analysis

A descriptive analysis of clinical variables, doses, and tacrolimus levels was performed. Qualitative data were described in relative and absolute frequencies, and quantitative data in measures

of central tendency and dispersion according to their distribution. Normal distribution was assessed by Kolmogorov-Smirnoff and Shapiro-Wilk tests. Categorical variables were compared by chi- and Mann Whitney U test for numerical variables. The incidence of rejection and toxicity at one-year post-transplantation of the included patients was described.

The correlation between tacrolimus levels and doses was evaluated by means of Spearman's correlation index considering a value of 0 - 0.25 as little or no correlation, 0.26-0.50 weak, 0.51-0.75 between moderate to strong and 0.76- 1.00 between strong to perfect. The categorizations of tacrolimus blood levels and dose were compared by mosaic plot, Cramer's v and chi-square. Finally, unadjusted Odds Ratios and confidence intervals (95%) were calculated for rejection by a logistic regression. A p-value of less than 0.05 was considered statistically significant. All analyses were performed in R Studio 3.3.0.

2.4. Ethical aspects

All transplants performed were in accordance with the Istanbul Declaration for organ donation, as well as national and international research guidelines. According to resolution 8430 of 1993, the study is a risk-free study, which is why it was exempted by the ethics committee from informed consent (Approval No. 01865).

3. RESULTS

We analyzed 368 kidney transplant patients, most had cadaveric donor transplantation (64.4%), with a median age of 44 years (RIC 33-56), weight of 68 kg (RIC 57-77) and a male population of 57.6%. The overall average tacrolimus levels during the initial two weeks post-transplantation were 4.98 ng/ml and showing an upward trend over subsequent months: 5.93 ng/ml in the first month, 6.18 ng/ml in the third month, 7.55 ng/ml in the sixth month, and 6.77 ng/ml at the twelfth month. On average, patients underwent 1.83 tacrolimus level assessments during their first-year post-transplant. Among the patients evaluated, 15.5% presented acute graft rejection and only 3.8% toxicity. There were not significant differences in rejection when comparing the added immunosuppression maintenance with mycophenolate and prednisolone. The detailed analysis is presented in Tables 1 and 2.

Table 1. Descriptive analysis of clinical and pharmacological characteristics and incidence of the main outcomes.

	Total (N=368)
Age (years), *Median [IQR]*	44.0 [33.0, 56.0]
Sex, *n(%)*	
Femenine	156 (42.4%)
Masculine	212 (57.6%)
Donor type, *n(%)*	
Deceased	237 (64.4%)
Living non-related	31 (8.4%)
Living related	100 (27.2%)
Weight (kg/m2), *Median [IQR]*	68.0 [57.0, 77.0]
Missing data	3 (0.8%)
Tacrolimus level first 2 weeks (ng/ml), *Median [IQR]*	4.20 [2.70, 6.23]
Missing data	144 (39.1%)
Maximum tacrolimus level (ng/ml), *Median [IQR]*	5.90 [4.50, 8.80]
Missing data	33 (9.0%)
Minimum tacrolimus level (ng/ml), *Median [IQR]*	3.80 [2.70, 5.85]
Missing data	33 (9.0%)
Number of tacrolimus levels, *Median [IQR]*	2.00 [1.00, 2.00]
Tacrolimus daily dose adjusted by weight, *Median [IQR]*	6.80 [5.70, 7.70]
Maximum tacrolimus daily dose (mg), *Median [IQR]*	5.00 [4.00, 6.00]
Missing data	11 (3.0%)
Minimum tacrolimus daily dose (mg), *Median [IQR]*	3.00 [2.00, 4.00]
Missing data	11 (3.0%)
Toxicity, *n(%)*	14 (3.8%)
Acute rejection, *n(%)*	57 (15.5%)
Banff patological clasification, *n(%)*	
1A	33 (9.0%)
1B	11 (3.0%)
2A	1 (0.3%)
Borderline	10 (2.7%)
Non-classified	2 (0.5%)
Non-biopsy	311 (84.5%)

Table 2. Bivariate analysis of rejection at the first, third, sixth and twelfth month and different clinical and pharmacological variables.

	1 MONTH GRAFT REJECTION			3 MONTH GRAFT REJECTION			6 MONTH GRAFT REJECTION			12 MONTH GRAFT REJECTION			TOTAL
	No	Yes	P value	No	Yes	P value	No	Yes	P value	No	Yes	P value	(N=368)
	(n=335)	(n=33)		(n=361)	(n=7)		(n=361)	(n=7)		(n=362)	(n=6)		
Age (years), *Median [IQR]*	44.0 [33.5, 56.0]	39.0 [30.0, 51.0]		44.0 [33.0, 56.0]	47.0 [37.5, 56.5]	0.951	44.0 [33.0, 56.0]	42.0 [37.5, 50.0]	0.962	44.0 [33.3, 56.0]	34.5 [29.3, 39.8]	0.199	44.0 [33.0, 56.0]
Sex, *n(%)*													
Femenine	143 (42.7%)	13 (39.4%)	0.935	153 (42.4%)	3 (42.9%)	1	154 (42.7%)	2 (28.6%)	0.757	152 (42.0%)	4 (66.7%)	0.479	156 (42.4%)
Masculine	192 (57.3%)	20 (60.6%)		208 (57.6%)	4 (57.1%)		207 (57.3%)	5 (71.4%)		210 (58.0%)	2 (33.3%)		212 (57.6%)
Donor type, *n(%)*													
Deceased	217 (64.8%)	20 (60.6%)	0.935	232 (64.3%)	5 (71.4%)	0.941	231 (64.0%)	6 (85.7%)	0.819	233 (64.4%)	4 (66.7%)	0.946	237 (64.4%)
Living non-related	29 (8.7%)	2 (6.1%)		30 (8.3%)	1 (14.3%)		31 (8.6%)	0 (0%)		30 (8.3%)	1 (16.7%)		31 (8.4%)
Living related	89 (26.6%)	11 (33.3%)		99 (27.4%)	1 (14.3%)		99 (27.4%)	1 (14.3%)		99 (27.3%)	1 (16.7%)		100 (27.2%)
Weight (kg/m2), *Median [IQR]*	68.0 [57.0, 77.0]	68.0 [56.0, 79.0]	0.919	68.0 [57.0, 77.0]	59.5 [54.0, 67.3]	0.894	68.0 [57.0, 77.0]	68.0 [57.0, 79.0]	0.995	68.0 [57.0, 77.0]	61.8 [55.1, 76.3]	0.947	68.0 [57.0, 77.0]
Missing data	3 (0.9%)	0 (0%)		3 (0.8%)	0 (0%)		3 (0.8%)	0 (0%)		3 (0.8%)	0 (0%)		3 (0.8%)
Tacrolimus level (ng/ml), *Median [IQR]*	5.40 [3.70, 7.50]	5.05 [2.98, 6.38]	0.497	5.40 [3.60, 7.70]	5.65 [4.39, 7.18]	0.987	7.00 [4.58, 9.10]	6.35 [6.13, 6.58]	0.934	7.30 [5.30, 8.00]	5.10 [4.40, 5.80]	0.544	5.30 [3.50, 7.50]
Missing data	152 (45.4%)	7 (21.2%)		297 (82.3%)	3 (42.9%)		351 (97.2%)	5 (71.4%)		357 (98.6%)	4 (66.7%)		159 (43.2%)
Tacrolimus level clasification, *n(%)*													
Low	77 (23.0%)	12 (36.4%)	0.974	26 (7.2%)	2 (28.6%)	0.983	3 (0.8%)	0 (0%)	0.499	1 (0.3%)	0 (0%)	0.545	89 (24.2%)
High	39 (11.6%)	4 (12.1%)		12 (3.3%)	1 (14.3%)		4 (1.1%)	0 (0%)		4 (1.1%)	1 (16.7%)		43 (11.7%)
Optimal	67 (20.0%)	10 (30.3%)		26 (7.2%)	1 (14.3%)		3 (0.8%)	2 (28.6%)		0 (0%)	1 (16.7%)		77 (20.9%)
Missing data	152 (45.4%)	7 (21.2%)		297 (82.3%)	3 (42.9%)		351 (97.2%)	5 (71.4%)		357 (98.6%)	4 (66.7%)		159 (43.2%)
Tacrolimus daily dose (mg), *Median [IQR]*	4.00 [4.00, 4.00]	4.00 [4.00, 4.00]	0.999	4.00 [4.00, 6.00]	4.00 [2.50, 4.50]	0.268	4.00 [4.00, 6.00]	4.00 [3.50, 4.00]	0.742	4.00 [3.00, 5.00]	6.50 [5.25, 7.75]	**0.0206***	4.00 [4.00, 4.00]
Missing data	11 (3.3%)	0 (0%)		31 (8.6%)	0 (0%)		69 (19.1%)	0 (0%)		99 (27.3%)	0 (0%)		11 (3.0%)

Diference weight adjusted and formulated dose (mg), *Median [IQR]*	2.70 [1.60, 3.60]	2.80 [1.40, 3.90]	0.913	2.10 [0.625, 3.90]	2.10 [0.725, 4.33]	1	2.08 [0.375, 3.95]	3.10 [2.30, 4.40]	0.995	2.70 [0.900, 4.40]	-0.225 [-1.50, 0.863]	0.511	2.70 [1.60, 3.60]
Missing data	11 (3.3%)	0 (0%)		31 (8.6%)	0 (0%)		69 (19.1%)	0 (0%)		99 (27.3%)	0 (0%)		11 (3.0%)
Micophenolate daily dose (mg), *Median [IQR]*	2000 [2000, 2000]	2000 [2000, 2000]		2000 [1500, 2000]	1500 [1500, 2000]		1500 [1000, 2000]	1500 [750, 2000]		1500 [1000, 2000]	1750 [1500, 2000]		2000 [2000, 2000]
Non-micophenolate maintance	11 (3.3%)	0 (0%)		47 (13.0%)	0 (0%)		88 (24.4%)	0 (0%)		88 (24.3%)	0 (0%)		11 (3.0%)
Coticoid maintenance, *n(%)*	33 (9.9%)	7 (21.2%)	0.135	50 (13.9%)	0 (0%)	0.571	50 (13.9%)	1 (14.3%)	0.999	41 (11.3%)	2 (33.3%)	0.25	40 (10.9%)
Prednisolone daily dose (mg), *Median [IQR]*	5.00 [5.00, 5.00]	5.00 [5.00, 5.00]	0.539	5.00 [5.00, 5.00]	NA	1	5.00 [5.00, 5.00]	5.00 [5.00, 5.00]	0.926	5.00 [5.00, 5.00]	5.00 [5.00, 5.00]	0.929	5.00 [5.00, 5.00]

*Statistically significant with p-value less than 0.05

3.1. Tacrolimus levels and dose correlation

The results revealed that there was no statistical significance between tacrolimus blood level categories and the doses of tacrolimus during the first twelve months. Moreover, tacrolimus blood level categories (decreased, optimal and elevated) presented very similar average doses in every assessment, mostly above 4 mg per day. However, it was observed that when tacrolimus blood levels were decreased, the doses between levels increased, and for elevated blood levels, the doses decreased. The results further revealed a weak correlation (rho=0.31, p <0.001) between the variability of levels and dose, indicating a directly proportional relationship (Figure 1). The complete analysis for the first, third, sixth, and twelfth months can be found in Appendix 1.

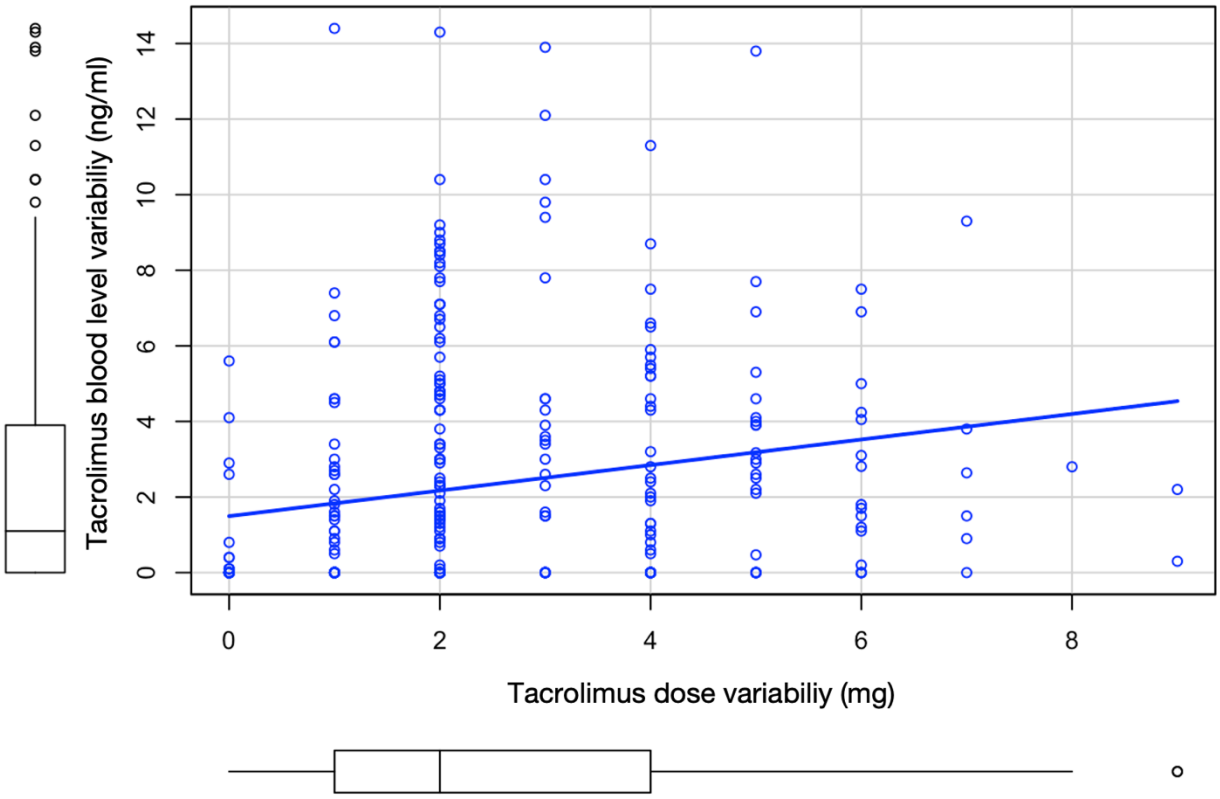

Figure 1. Correlation between tacrolimus blood levels and dose variability.

3.2. Tacrolimus levels and graft rejection association

The results did not reveal statistically significant differences in the bivariate analysis and non-adjusted logistic regression for tacrolimus levels and doses with acute graft rejection. Except for the twelfth-month tacrolimus doses, which were higher in the patients that presented acute rejection the same month (p-value 0.02). The p values for each test are presented in Table 2, and the non-adjusted regression is in Appendix 1.

4. DISCUSSION

Currently, monitoring tacrolimus levels is still considered a useful and decisive tool for individualizing patient treatment; however, the usefulness of monitoring these levels is still unclear. Different studies support a wide and variable range of conclusions. A cohort study in the United States stated that there is an association between tacrolimus levels and biopsy-proven acute rejection, specifically in levels below eight ng/ml [9]. In contrast, another cohort study in Peru found no link between tacrolimus levels and renal function and acute rejection but found that levels higher than 12 ng/ml were associated with early infections in the first post-transplant month [10]. An additional study, although it asserts the absence of an association between levels and rejection, pointing out the limitation in the number of patients included as an influential factor [11]. The present study suggests that tacrolimus levels taken during the first-year post-transplantation and the incidence of graft rejection during this same period are not significantly related; even the number of levels taken, and maximum/minimum levels do not represent a decrease or increase in rejection rates.

Furthermore, populations with equivalent therapies, such as double therapy composed of tacrolimus and mycophenolate, do not result in statistically significant changes in acute rejection. This same result was present in patients with triple therapies, including prednisolone, demonstrating a lack of protection against the incidence of graft rejection. This supports the double therapy immunosuppression maintenance that reduces the possible adverse effects of chronic glucocorticoid use, including diabetes, Cushing syndrome, osteoporosis, and hypertension, among many others [12].

Moreover, the data obtained shows a statistically significant correlation between tacrolimus levels and dose variability but failed to present a significant correlation between each month's doses and levels. We consider these results confirm the relevance of tacrolimus personal variability, which can be related to genetic factors, food, medication, concomitant illnesses, and adherence [13]. In fact, several papers are studying the intra-patient tacrolimus variability and its association to rejection and toxicity [13-16]. Nonetheless, it is still recommended the tacrolimus blood level, especially in the first few weeks after transplantation [17]. This timing is consistent with this study since the greatest number of levels were obtained during the first two weeks and the first month post-transplantation despite the significant percentage of missing data.

When analyzing tacrolimus blood levels by categories, no significant differences were found when comparing the formulated doses. However, it was noticed that in most cases where the levels were decreased, the dose increased, and when the levels were high, the dose decreased. This suggests that there may be a clinical response to tacrolimus levels. But it is crucial to note that, as stated in a study, tacrolimus concentration variations do not provide a clear association or guideline for dose reduction over time [18].

Another significant finding was the relatively low occurrence of toxicity, affecting less than 4% of patients. This outcome could be attributed to two factors: firstly, the limited sample size, which has been observed in other studies investigating these phenomena [14]. Secondly, there may have been an underreporting of toxicity events, considering that the identification of toxicity was based on the review of the patients' clinical histories, since there have not been any report adverse events to the pharmacovigilance group. These observations align with existing literature that indicates a lack of feeding of the system by health professionals, highlighting the need to

identify and work on the causes of underreporting to improve patient safety [19]. It would be worthwhile to review if the underreporting diminishes if the pharmacovigilance group should define tacrolimus toxicity and how it relates to drug doses and levels. On the other hand, it is important to note that Colombiana de Trasplantes uses reduced tacrolimus doses, which may have an influence on tacrolimus levels and toxicity events. Therefore, it would be worthwhile to study reduced doses and total compliance and knowledge of toxicity report with a larger population.

This study is, to our knowledge, the first report on tacrolimus levels and doses, rejection, and toxicity in Colombia. But must be understood within its limitations; first, the missing data regarding tacrolimus levels caused by hospitalization, absence of a request, nephrectomy, graft loss, and death. This was especially true during the first month when all patients were expected to have their levels taken as per the immunosuppression guide.

Additionally, there were significant differences in the level results obtained in short periods, possibly due to various factors such as the technique used in taking the levels, patient taking medication before the test, drug interactions, or the time between taking the sample and the reading. The technique is particularly relevant as the analytical methodology used can be influenced by significant cross-reactivity of metabolites or structural analogues, sample collection time and measurement not only of the free drug but also of the protein-bound drug which, although taken into account, is an important factor to be considered [20, 21]. Second, the non-randomized and small sample size may limit the generalization of these results. Finally, a suspected underreport of the tacrolimus toxicity may affect the incidence of this event.

Finally, a totally influential factor for the type of treatment of renal transplant patients that plays a fundamental role in graft rejection rates is adherence, an aspect that can also influence tacrolimus levels in the event of incorrect administration or lack thereof [22, 23]. As for toxicity, defining the association with the drug continues to be a challenge both for the specialist and for the pharmacovigilance teams since due to the complexity of the treatment and the pathology, there may be other factors that can explain it, such as concomitant drugs or other pathologies, in addition to the absence of information related to it, and the wide range of adverse reactions detailed in the drug's technical data sheet, do not allow for a clear consensus on toxicity associated with tacrolimus.

Among the challenges are the need for further studies according to each factor studied, encouraging the reporting of adverse events that allow grouping and defining toxicity, digital aids that allow easy and complete clinical information on the patient, in addition to the search for other tools, such as pharmacogenetics, which can be both a clinical and economic tool for defining immunosuppressive therapy for patients [7].

5. CONCLUSIONS

In conclusion our study did not find a significant association of tacrolimus blood levels with graft rejection in the first year. Even so, we consider that tacrolimus levels are relevant for the kidney recipient care, but are highly variable between doses, adherence, and pharmacodynamics. On the other side, we found that low toxicity incidence did not vary upon tacrolimus levels but suspect that this phenomenon is affected by underreporting. Emerging the question of the usefulness and

relevance of tacrolimus plasma level monitoring as an effective and determinant approach to mitigate rejection or toxicity rates. Therefore, to answer there is a need to scale this study to a bigger sample size and probably including novel assessments including pharmacogenetic factors, aiming to have a better understanding of the pharmacokinetics in our population and to encourage the adequate report of suspected toxicity events.

CONFLICT OF INTEREST

All authors report that they do not have any conflicts of interest.

APPENDIX 1.

Table A1. Tacrolimus blood level categorization in the first month

	Decreased (N=89)	Elevated (N=43)	Optimal (N=77)	Total (N=209)	P value
Tacrolimus daily dose - Fist month (mg)					
Mean (SD)	4.07 (0.636)	4.05 (0.375)	4.08 (0.390)	4.07 (0.505)	0.991
Median [Q1, Q3]	4.00 [4.00, 4.00]	4.00 [4.00, 4.00]	4.00 [4.00, 4.00]	4.00 [4.00, 4.00]	
Difference weight adjusted and formulated dose (mg)					
Mean (SD)	2.50 (1.46)	2.82 (1.41)	2.70 (1.34)	2.64 (1.40)	0.639
Median [Q1, Q3]	2.60 [1.50, 3.50]	2.90 [1.85, 3.60]	2.70 [1.90, 3.50]	2.70 [1.60, 3.50]	

Table A2. Tacrolimus blood level categorization in the first and third month

	Decreased (N=28)	Elevated (N=13)	Optimal (N=27)	Total (N=68)	P value
Tacrolimus daily dose - third month (mg)					
Mean (SD)	5.00 (1.83)	3.77 (1.24)	4.22 (1.45)	4.46 (1.63)	0.111
Median [Q1, Q3]	5.00 [4.00, 6.00]	4.00 [3.00, 4.00]	4.00 [4.00, 5.00]	4.00 [4.00, 6.00]	
Tacrolimus daily dose - first month (mg)					
Mean (SD)	4.14 (0.932)	4.15 (0.555)	3.93 (0.385)	4.06 (0.689)	0.645
Median [Q1, Q3]	4.00 [4.00, 4.00]	4.00 [4.00, 4.00]	4.00 [4.00, 4.00]	4.00 [4.00, 4.00]	
Difference weight adjusted and formulated dose (mg)					
Mean (SD)	1.36 (2.53)	3.24 (2.09)	2.70 (2.15)	2.25 (2.40)	0.0672
Median [Q1, Q3]	0.950 [-0.500, 3.05]	3.60 [2.10, 4.50]	2.60 [1.40, 4.50]	2.55 [0.713, 4.15]	

Table A3. Tacrolimus blood level categorization in the third and sixth month

	Decreased (N=3)	Elevated (N=4)	Optimal (N=5)	Total (N=12)	P value
Tacrolimus daily dose - sixth month (mg)					
Mean (SD)	5.67 (1.53)	3.50 (1.00)	4.60 (2.19)	4.50 (1.78)	0.47
Median [Q1, Q3]	6.00 [5.00, 6.50]	4.00 [3.50, 4.00]	4.00 [4.00, 5.00]	4.00 [4.00, 5.25]	
Tacrolimus daily dose - third month (mg)					
Mean (SD)	5.33 (1.15)	4.00 (0)	3.20 (1.64)	4.00 (1.41)	0.22
Median [Q1, Q3]	6.00 [5.00, 6.00]	4.00 [4.00, 4.00]	4.00 [2.00, 4.00]	4.00 [4.00, 4.25]	
Difference weight adjusted and formulated dose (mg)					
Mean (SD)	0.900 (2.70)	3.65 (2.13)	2.53 (3.22)	2.50 (2.74)	0.642
Median [Q1, Q3]	-0.400 [-0.650, 1.80]	3.33 [1.98, 5.00]	2.80 [2.30, 4.70]	2.55 [1.21, 4.63]	

Table A4. Tacrolimus blood level categorization in the twelfth month

	Decreased (N=1)	Elevated (N=1)	Optimal (N=5)	Total (N=7)	P value
Tacrolimus daily dose - twelfth month (mg)					
Mean (SD)	NA	NA	4.20 (1.64)	4.29 (1.38)	0.961
Median [Q1, Q3]	5.00 [5.00, 5.00]	4.00 [4.00, 4.00]	4.00 [3.00, 4.00]	4.00 [3.50, 4.50]	
Difference weight adjusted and formulated dose (mg)					
Mean (SD)	NA	NA	3.09 (1.29)	2.95 (1.12)	0.878
Median [Q1, Q3]	3.15 [3.15, 3.15]	2.05 [2.05, 2.05]	3.30 [2.75, 3.80]	3.15 [2.40, 3.55]	

Table A5. Non adjusted Odds Ratios (OR), confidence intervals (CI) and p value of graft rejection at the first, third, sixth and twelfth.

	1 MONTH GRAFT REJECTION			3 MONTH GRAFT REJECTION			6 MONTH GRAFT REJECTION			12 MONTH GRAFT REJECTION		
	OR	**CI 95%**	**P value**	**OR**	**CI 95%**	**P value**	**OR**	**CI 95%**	**P value**	**OR**	**CI 95%**	**P value**
Age *(years)*	0.98	0.95-1.00	0.180	1.00	0.95-1.06	0.750	0.99	0.94-1.04	0.780	0.94	0.88-1.00	0.083
Sex *(Reference Feminine)*												
Masculine	1.14	0.55-2.43	0.715	0.98	0.21-5.03	0.98	1.85	0.39-13.1	0.462	0.36	0.04-1.87	0.244
Donor type *(Reference Deceased)*												
Living non-related	0.74	0.11-2.74	0.706	1.54	0.07-10.02	0.695	Inf	NC	0.99	1.94	0.09-13.66	0.559
Living related	1.34	0.59-2.86	0.459	0.468	0.024		0.38	0.02-2.31	0.385	0.58	0.02-4.03	0.637

Weight (kg/m^2)	0.99	0.97-1.00	0.700	0.97	0.92-1.005	0.313	0.99	0.95-1.00	0.920	0.98	0.93-1.00	0.579
Tacrolimus level (ng/ml)**	0.91	0.78-1.04	0.237	0.97	0.64-1.29	0.868	0.91	0.50-1.26	0.699	0.46	0.05-1.24	0.284
Tacrolimus level classification *(Reference Optimal)*												
Decreased	1.04	0.42-2.62	0.925	2.00	0.18-44.6	0.580	Inf	NC	0.998	Inf	NC	0.998
Elevated	0.68	0.17-2.20	0.548	2.16	0.08-57.9	0.595	Inf	NC	0.998	Inf	NC	0.999
Tacrolimus daily dose *(mg)***	1.01	0.49-1.80	0.964	0.65	0.37-1.07	0.105	0.83	0.50-1.28	0.438	1.58	1.11-2.28	**0.008***
Difference weight adjusted and formulated dose (mg)**	0.97	0.78-1.04	0.691	1.00	0.76-1.06	0.998	1.00	0.80-1.06	0.919	0.68	0.49-0.93	**0.017***
Mycophenolate (Reference No)												
Mofetil	Inf	NC	0.990	Inf	NC	0.992	Inf	NC	0.993	Inf	NC	0.993
Sodium				Inf	NC	1	1	NC	1	Inf	NC	1
Corticoid maintenance *(Reference No)*												
Yes	2.46	0.92-5.85	0.051	1.41	NC	0.992	1.03	0.05-6.24	0.974	3.91	0.53-20.7	0.122
Prednisolone daily dose (mg)**	0.51	0.13-1.24	0.230	Inf	NC	1	45.5	NC	0.997	Inf	NC	0.996

*Statistically significant with p-value less THAN 0.05

** Taken the same month of the graft rejection

REFERENCES

1. T. Vos, C. Allen, M. Arora, R.M. Barber, A. Brown, A. Carter, *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*, **388**(10053), 1545–1602 (2016). Doi: http://doi.org/10.1016/S0140-6736(16)31678-6
2. F. Giron-Luque, N. Patino-Jaramillo, Y. Baez-Suarez, A. García-López, F. Giron-Luque, N. Patino-Jaramillo, *et al.* The use of intraoperative mannitol during the laparoscopic nephrectomy in living kidney donor and the prevention of delayed graft function. *Urol. Colomb.*, **32**(3), 86–92 (2023). Doi: http://doi.org/10.24875/ruc.23000029
3. D.F. Ávila, M. Caicedo, S.J. Echeverry, A.F. Bohórquez, P.K. García, M.A. Nova, *et al. Posicionamiento terapéutico para el tratamiento farmacológico de trasplante de riñón*. Instituto de Evaluación Tecnológica en Salud, Ministerio de Salud y Protección Social, Bogotá D. C., Colombia, 2021, 388 p. URL: https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/VP/RBC/ipt-trasplante-organo-solido-rinon-2021.pdf. Accessed on July 16, 2023.
4. E. Peña-Torres & S. Narváez. *Efectividad y seguridad de tacrolimus como inmunosupresión primaria en pacientes con trasplante renal*. Instituto de Evaluación Tecnológica en Salud, Ministerio de Salud y Protección Social, Bogotá D. C., Colombia, 2013, 28 p. URL: https://www.iets.org.co/Archivos/EyS%20Trasplante%20renal%20(tacrolimus).pdf. Accessed on July 16, 2023.

5. A.W. Thomson, C.A. Bonham & A. Zeevi. Mode of action of tacrolimus (FK506): Molecular and cellular mechanisms. *Ther. Drug Monit.*, **17**(6), 584–591 (1995). Doi: http://doi.org/10.1097/00007691-199512000-00007
6. INVIMA. Listado de principios activos de estrecho margen terapéutico, 2017. URL: https://www.invima.gov.co/documents/20143/1232250/Listado-de-Medicamentos-de-Estrecho-Margen-Terapeuticos.pdf/b4c0b80a-e61e-a93a-8195-caca922f9cef?t=1560783097694. Accessed on July 16, 2023.
7. B. Tavira, C. Díaz-Corte, D. Coronel, F. Ortega & E. Coto. Farmacogenética del tacrolimus: ¿del laboratorio al paciente? *Nefrologia*, **34**(1), 11–17 (2014). Doi: http://doi.org/10.3265/nefrologia.pre2013.nov.12267
8. S. Salcedo-Herrera, J.L. Pinto-Ramirez, A. García-Lopez, J. Amaya-Nieto & F. Girón-Luque. Acute rejection in kidney transplantation and early beginning of tacrolimus. *Transplant. Proc.*, **51**(6), 1758–1762 (2019). Doi: http://doi.org/10.1016/j.transproceed.2019.04.048
9. K.R. Richards, D. Hager, B. Muth, B.C. Astor, D. Kaufman & A. Djamali. Tacrolimus trough level at discharge predicts acute rejection in moderately sensitized renal transplant recipients. *Transplantation*, **97**(10), 986–991 (2014). Doi: http://doi.org/10.1097/TP.0000000000000149
10. M. Gómez-Luján, M.E. Chambi-Macedo, C. Cruzalegui-Gómez, H. Soto-Huamán, E. Sifuentes-Aguirre & J. Gálvez-Inga. Relationship between tacrolimus dosage and renal function, acute rejection and early infections, single center experience. *Rev. Fac. Med. Hum.*, **18**(2), 21–27 (2018). Doi: http://doi.org/10.25176/rfmh.v18.n2.1282
11. M.I. Francke, L.M. Andrews, H.L. Le, D. van de Velde, M. Dieterich, S. Udomkarnjananun, *et al.* Monitoring intracellular tacrolimus concentrations and its relationship with rejection in the early phase after renal transplantation. *Clin. Biochem.*, **101**, 9–15 (2022). Doi: http://doi.org/10.1016/j.clinbiochem.2021.12.002
12. J. Gutiérrez-Restrepo. Efectos adversos de la terapia con glucocorticoides. *Iatreia*, **34**(2), 137–150 (2021). Doi: http://doi.org/10.17533/udea.iatreia.96
13. H.M. Gonzales, J.W. McGillicuddy, V. Rohan, J.L. Chandler, S.N. Nadig, D.A. Dubay, *et al.* A comprehensive review of the impact of tacrolimus intrapatient variability on clinical outcomes in kidney transplantation. *Am. J. Transplant.*, **20**(8), 1969–1983 (2020). Doi: http://doi.org/10.1111/ajt.16002
14. L. Schumacher, A.D. Leino & J.M. Park, Tacrolimus intrapatient variability in solid organ transplantation: A multiorgan perspective. *Pharmacotherapy*, **41**(1), 103–118 (2021). Doi: http://doi.org/10.1002/phar.2480
15. G. Coste & F. Lemaitre. The role of intra-patient variability of tacrolimus drug concentrations in solid organ transplantation: A focus on liver, heart, lung and pancreas. *Pharmaceutics*, **14**(2), 379 (2022). Doi: http://doi.org/10.3390/pharmaceutics14020379
16. H. Kim, A. Han, S. Ahn, S.K. Min, J. Ha & S. Min. Association of high intra-patient variability in tacrolimus exposure with calcineurin inhibitor nephrotoxicity in kidney transplantation. *Sci. Rep.*, **13**, 16502 (2023). Doi: http://doi.org/10.1038/s41598-023-43755-x
17. K.L. Lentine, B.L. Kasiske, A.S. Levey, P.L. Adams, J. Alberú, M.A. Bakr, *et al.* KDIGO Clinical practice guideline on the evaluation and care of living kidney donors. *Transplantation*, **101**(8S), S7–S105 (2017). Doi: http://doi.org/10.1097/TP.0000000000001769
18. A.L. Robles-Piedras & E.H. González-López. Tacrolimus levels in adult patients with renal transplant. *Proc. West Pharmacol. Soc.*, **52**, 33–34 (2009). URL: https://web.uaeh.edu.mx/investigacion/icsa/LI_UsoMedic/Ana_Robles/tacrolimus.pdf
19. O. Moreno-Mattar, R. Ramírez, A. Alvarado, F. Sierra & J. Guzmán. Report of adverse events in Colombia: A successful case. *Curr. Drug Saf.*, **14**(1), 53–56 (2018). DOI: http://doi.org/10.2174/1574886313666181109150648

20. R. Gómez-Lagos. *Recomendaciones para la cuantificación de inmunosupresores en el monitoreo de drogas terapeúticas*. Instituto de Salud Pública, Ministerio de Salud, Santiago, Chile, 2017, 22 p. URL: https://www.ispch.cl/sites/default/files/Recomendaciones%20para%20la%20cuantificaci%C3%B3n%20de%20inmunosupresores.pdf
21. Y.-W. Lee. Comparison between ultra-performance liquid chromatography with tandem mass spectrometry and a chemiluminescence immunoassay in the determination of cyclosporin A and tacrolimus levels in whole blood. *Exp. Ther. Med.*, **6**(6), 1535–1539 (2013). Doi: http://doi.org/10.3892/etm.2013.1325
22. M. Torres-Gutiérrez, N. Lozano-Suárez, V.A. Burgos-Camacho, J. Caamaño-Jaraba, J.A. Gómez-Montero, A. García-López, *et al.* Is non-adherence associated with adverse outcomes in kidney transplant recipients? The role of non-adherence as a risk and predictor factor for graft loss and death. *Patient Prefer. Adherence*, **17**, 2915–2925 (2023). Doi: http://doi.org/10.2147/ppa.s436833
23. M. Torres-Gutiérrez, V. Burgos-Camacho, J.P. Caamaño-Jaraba, N. Lozano-Suárez, A. García-López & F. Girón-Luque. Prevalence and modifiable factors for holistic non-adherence in renal transplant patients: A cross-sectional study. *Patient Prefer. Adherence*, **17**, 2201–2213 (2023). Doi: http://doi.org/10.2147/ppa.s419324

HOW TO CITE THIS ARTICLE

N. Gaitán-Torres, N. Lozano-Suárez, A. García-López, A. Gómez-Montero & F. Girón-Luque. Correlating Tacrolimus levels with dose formulation: Implications for toxicity and renal graft rejection in a cohort of kidney transplant recipients with steroid-free maintenance immunosuppression. *Rev. Colomb. Cienc. Quim. Farm.*, **54**(1), 28–43 (2025). Doi: https://doi.org/10.15446/rcciquifa.v54n1.113036