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ORIGINAL ARTICLE

Description of genetic polymorphisms in CYP3A5 and MDR-1 and their impact on clinical acute rejection in liver transplant patients at Hospital San Vicente Fundación Rionegro

Descripción de polimorfismos genéticos en CYP3A5 y MDR-1 y su impacto en el rechazo agudo clínico de pacientes trasplantados de hígado del Hospital San Vicente Fundación Rionegro

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Abstract

Introduction. Tacrolimus is an immunosuppressive drug widely used in liver transplantation, which presents great interindividual variability, which is considered associated with the frequency of CYP3A5 and MDR-1 polymorphisms. The objective of this study was to evaluate the frequency of the rs776746, rs2032582 and rs1045642 polymorphisms and their association with clinical rejection and drug toxicity.

Methods. Seventeen immunosuppressed patients with tacrolimus who underwent a liver transplant at the Hospital San Vicente Fundación Rionegro between 2020 and 2022 were included, with survival of more than one month. Clinical variables, acute rejection and pharmacological toxicity were evaluated. The study genes were sequenced by PCR, comparing their expression or not in each of the patients.

Results. 43% of the patients were classified as CYP3A5*1/*1 and CYP3A5*1/*3, among which an association was found with increased rates of clinical acute rejection when compared with non-expressive patients (100% vs. 44%, p=0.05). There were no differences in drug toxicity or other outcomes. The rs2032582 polymorphism was found in 50% and rs1045642 in 23.5% of patients; however, no association with rejection or other clinical events was identified.

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Conclusions. An association was found between the CYP3A5*1/*1 and CYP3A5*1/*3 genotype and the clinical rejection rate. However, a larger sample is required to validate these data and propose models of personalized medicine.

Keywords: organ transplantation; liver transplantation; graft rejection; single nucleotide polymorphism; Tacrolimus; pharmacogenetics.

Resumen

Introducción. El tacrolimus es un medicamento inmunosupresor ampliamente usado en trasplante hepático, que presenta una gran variabilidad interindividual la cual se considera asociada a la frecuencia de polimorfismos de CYP3A5 y MDR-1. El objetivo de este estudio fue evaluar la frecuencia de los polimorfismos rs776746, rs2032582 y rs1045642 y su asociación con rechazo clínico y toxicidad farmacológica.

Métodos. Se incluyeron 17 pacientes inmunosuprimidos con tacrolimus a quienes se les realizó trasplante hepático en el Hospital San Vicente Fundación Rionegro entre 2020 y 2022, con supervivencia mayor a un mes. Se evaluaron las variables clínicas, rechazo agudo y toxicidad farmacológica. Se secuenciaron los genes de estudio mediante PCR, comparando la expresión o no en cada uno de los pacientes.

Resultados. El 43 % de los pacientes se clasificaron como CYP3A5*1/*1 y CYP3A5*1/*3, entre los cuales se encontró asociación con aumento en la tasa de rechazo agudo clínico, al comparar con los pacientes no expresivos (100 % vs. 44 %, p=0,05); no hubo diferencias en cuanto a la toxicidad farmacológica u otros desenlaces. Se encontró el polimorfismo rs2032582 en un 50 % y el rs1045642 en un 23,5 % de los pacientes, sin embargo, no se identificó asociación con rechazo u otros eventos clínicos.

Conclusiones. Se encontró una asociación entre el genotipo CYP3A5*1/*1 y CYP3A5*1/*3 y la tasa de rechazo clínico. Sin embargo, se requiere una muestra más amplia para validar estos datos y plantear modelos de medicina personalizada.

Palabras clave: trasplante de órganos; trasplante de hígado; rechazo de injerto; polimorfismo de nucleótido simple; Tacrolimus; farmacogenética.

Introduction

Liver transplantation is the last step in the treatment of end-stage liver disease, acute liver failure and some primary liver neoplasms [1]. Currently, tacrolimus, an immunosuppressive drug that inhibits calcineurin, is the drug of choice to prevent graft rejection [2,3]. Its mechanism of action consists of inhibiting the production of interleukin 2 in the CD4+ T lymphocyte [4]. It is considered to have a narrow therapeutic window and a large inter- and intra-individual pharmacokinetic variation, which makes the implementation of an empirical dosing protocol difficult and implies the constant measurement of its levels to guarantee the therapeutic effect and reduce the incidence of adverse effects such as nephrotoxicity, neurotoxicity and hyperglycemia [5].

Tacrolimus is absorbed in the small intestine via P-glycoprotein or MDR-1, which is encoded by the ABCB1 gene, an efflux transporter located on the enterocyte membrane, which negatively regulates drug absorption [4]. Within the intestinal cell, the gastrointestinal isoenzymes CYP3A4 and CYP3A5 carry out an initial metabolism and the secondary metabolites of this process are eliminated through the stool. This, combined with first-order hepatic metabolism, explains why after oral administration there is poor bioavailability, close to 25% [6]. The metabolism of tacrolimus is hepatic and is carried out by the cytochromes

CYP3A4 and CYP3A5, which generate approximately 15 metabolites, some to a lesser extent, with an immunosuppressive activity similar to the original drug; 95% of its metabolites are eliminated via the bile and 2% via the urine [7].

Drug and food interactions and interindividual variability in drug dosage are influenced by the activity of cytochrome P450-3A5 [4]. Single nucleotide polymorphism (SNP) within intron 3 of CYP3A5 has been a topic of study. Homozygous carriers (called extensive metabolizers) or heterozygous carriers (intermediate metabolizers) for the CYP3A5*1 wild-type allele produce higher levels of the functional protein, which has been associated with increased drug dose requirements, when compared with patients who carry two copies of the CYP3A5*3 variant allele (poor metabolizers), in which the result is a truncated enzyme with poor function [8].

Approximately 80% of Caucasians are homozygous for the CYP3A5*3 variant allele and the majority of black patients are homozygous for the wild-type allele [4]. The 3435C>T polymorphisms in exon 26 and the 2677G>T/A in exon 21 of the ABCB1 gene, which encodes P-glycoprotein, are directly associated with mechanisms that generate low expression of intestinal MDR-1 or directly induce indirect decrease in transporter substrate specificity. Despite being a matter of controversy, it has been suggested that the presence of these mutations increases the bioavailability of the drug and thus reduces the required doses when compared to carriers of the wild gene [9]. It has been proposed that the implementation of individualized immunosuppression protocols based on the genotyping of the donor and recipient could be a strategy that helps reduce the variability in drug levels and, in the same way, the rates of adverse events, such as acute rejection and nephrotoxicity [10,11].

In Colombia, we currently do not identify studies that describe the frequency of these polymorphisms in our population, their impact on immunosuppression and the clinical outcomes of patients undergoing liver transplantation. The objective of this study was to describe the presence of genetic polymorphisms of the MDR1 gene (rs1045642 and rs2032582) and the CYP3A5*3 allele in the CYP3A5 gene in patients undergoing liver transplantation at the Hospital San Vicente Fundación Rionegro, to evaluate their relationship with clinical acute rejection and the incidence of adverse events.

Methods

Prospective and analytical observational study, which included patients over 18 years of age undergoing orthotopic liver transplantation at the Hospital San Vicente Fundación Rionegro, Colombia, between 2020 and 2022, who received tacrolimus for at least 1 month, with follow-up for more than 30 days, regardless of whether or not they received steroids or mycophenolate mofetil. Patients diagnosed with human immunodeficiency virus infection, pregnant or lactating women, or consuming contraceptives, and patients undergoing multiple organ transplants were excluded.

Immunosuppression

According to the hospital protocol, immunosuppression in its induction phase began intraoperatively with the administration of methylprednisolone 1 g IV prior to reperfusion. Immediately after the transplant, tacrolimus was started at a dose of 0.1 mg/kg/day every 12 hours and mycophenolate mofetil 1 g/12 hours. The IV steroid was progressively discontinued until the fourth day, when oral prednisolone 20 mg/day in a single dose was switched. According to the criteria of the treating hepatologist, in some cases a scheme of progressive introduction of tacrolimus was carried out with a starting dose of 0.04 to 0.06 mg/kg/day, in order to reduce the incidence of post-transplant acute kidney injury [12,13].

Monitoring plasma levels and outcomes

Plasma monitoring of the medication was done serially during the hospital stay. The first measurement was carried out between the fourth and fifth day after initiation and then until therapeutic levels between 6-10 ng/mL were achieved [3]. Patients who required formulation adjustments had additional measurements, as did patients

with persistent organ dysfunction or uncontrolled infections, who required them according to the hepatologist's criteria. The institutional outpatient follow-up protocol included weekly measurements in the first month, biweekly until the third month, and monthly thereafter. If the levels were not within the therapeutic window or in case of altered liver profile, additional tests were performed.

Study variables

From the clinical history, sociodemographic data were collected from both the donor and the recipient (date of birth, sex, weight and height), the indication for the transplant, the technique used (whole liver, partition or reduction), the length of stay in the Intensive Care Unit (ICU) and complications during follow-up. In addition, pharmacological information such as the dosage of tacrolimus, the use of concomitant immunosuppressants or additional medications previously reported in the literature with strong and moderate interactions. Laboratory parameters such as blood count, liver profile (aminotransferases, bilirubins, alkaline phosphatase and gamma glutamyl transferase), kidney function, albumin and INR were monitored.

The primary outcome was the presence of acute or early graft rejection as an indicator of the effectiveness of immunosuppressive therapy. This was defined as the elevation of canalicular enzymes or aminotransferases between 5-30 day post-transplant, in the absence of other documented vascular or biliary causes that would explain the condition [14]. Drug levels were evaluated in relation to the enzymatic peak associated with rejection and in patients who did not present rejection, levels were documented between 6-10 day after transplant. According to the previously mentioned objective levels, they were classified as infratherapeutic (<6 ng/mL), therapeutic (6-10 ng/mL) or supratherapeutic (>10 ng/mL), and were additionally correlated with the dose of the corresponding medication.

As secondary outcomes, the following adverse events were monitored during any time of follow-up:

- *Nephrotoxicity:* the presence of acute kidney injury was defined according to AKIN criteria [15]. Within this scenario, a differential evaluation of perioperative acute kidney injury was made, which presents with a pathophysiology and risk factors different from drug toxicity (sepsis, hemodynamic instability, reperfusion syndrome, polytransfusion) and was defined as the presence of acute kidney injury in the first seven postoperative days [16].
- *Hepatotoxicity:* also called drug-induced liver injury, it was defined as the presence of elevation of aminotransferases equal to or more than five times above normal upper limit, alkaline phosphatase two or more times above normal upper limit, or total bilirubin two or more times above normal upper limit [17].
- *Neurotoxicity:* it was defined as the presence of tremor, neuralgia, peripheral neuropathy, psychosis, hallucinations, visual acuity alterations, cerebellar ataxia or paresis [18].

A causal association with treatment was identified, for which the WHO-UMC evaluation was used, which classifies the association as certain, possible, probable, improbable, conditional or unclassified, and unclassifiable. Positive associations were considered those classified as certain, possible and probable [19].

*DNA extraction and genotyping of CYP3A5*3 and MDR1-C3435T and MDR1- G2677T*

Taking paraffin samples and DNA extraction: Paraffin tissue sampling was performed from four 10-micrometer-thick recipient liver biopsy sections. These liver biopsies were embedded in formaldehyde paraffin to later be stored. To extract genomic DNA from these samples, the MagMAX® FFPE DNA/RNA Ultra Kit (CAT A31881, Thermofisher) was used. To verify the quality and integrity of the extracted DNA, 1% agarose gels were used. The DNA was stored at -80°C until the genotyping or sequencing process.

Sanger sequencing: DNA concentration was measured using a NanoDrop spectrophotometer. Initially, allelic variants in the MDR1 gene such as

C3435T (rs1045642) and G2677T (rs2032582) and the CYP3A5*3 allele in the CYP3A5 gene were detected by PCR, thus confirming the presence of each DNA portion of interest. of the polymorphisms analyzed. These were amplified using primers designed by Macrogene. The extensions were subsequently sequenced using the Sanger sequencing platform on an Applied Biosystems 3500. The base calling, alignment and assembly of the consensus sequences and their editing were carried out with the Sequencher software accompanied by FinchTV V.1.4 and Bioedit V.7.2. Patients with the CYP3A5*1/*1 or CYP3A5*1/*3 genotype were classified as "expressive", while those with CYP3A5*3/*3 typing were classified as "non-expressive". Regarding the ABCB1-A gene (rs2032582), homozygotes for TT and heterozygotes (TG) were classified as "expressive", while homozygotes for GG were called "non-expressive". Finally, for the evaluation of the ABCB1-B gene (rs1045642), homozygotes for CC and heterozygotes (TC) were classified as "expressive", while homozygotes for TT were defined as "non-expressive".

Statistic analysis

Exploratory analysis: The clinical, biochemical, genetic and sociodemographic variables were expressed using proportions for nominal variables, mean and standard deviation for continuous variables. Continuous variables without normal distribution were expressed as median and range.

Analytical phase: Qualitative and quantitative variables were analyzed. Because it was a small sample and did not meet normality criteria, non-parametric tests were used for the inferential analysis, the Wilcoxon test for quantitative variables and the Fisher test for qualitative variables, both with a significance level of 0.05. Statistical analysis was performed with the R program version 3.5.0.

Results

Between 2020 and 2022, 34 liver transplants were performed. Eight patients who died in the first six months and who never received tacrolimus and one patient with a two-year follow-up who was treated with cyclosporine were excluded from the study. Eight patients with insufficient samples for the genetic study of DNA integrity were subsequently excluded, so 17 patients were finally included in the study (Table 1).

No statistically significant difference was found between baseline sociodemographic factors, the severity of liver disease prior to transplantation, tacrolimus levels during the time of rejection or during day 6-10 in patients without clinical acute rejection or in the incidence of perioperative acute kidney injury. Acute clinical rejection occurred more frequently in expressive patients (100% vs 44%, p=0.05) (Table 2).

It was possible to amplify and sequence the SNP called ABCB1-A (rs2032582) in 12 patients, the ABCB1-B SNP (rs1045642) in 17 and the CYP3A5 SNP (rs776746) in 16, taking into account that in ABCB1-A the wild genotype is homozygous for thymine, in ABCB1-B it is homozygous for cytosine and, as described above, the normal genotype encoding CYP3A5 corresponds to two copies of the wild-type or CYP3A5*1/*1 gene (Table 3).

The results of the univariate comparative analysis between the groups called "expressive" and "non-expressive" according to the genotyping of ABCB1-B (rs1045642), that is, one of the P-glycoprotein study polymorphisms, are presented in the table 4. No differences were found in terms of age, sex, Child-Pugh or MELD score, tacrolimus levels in the first 10 days, the presence of subtherapeutic levels associated with rejection, perioperative acute kidney injury or the clinical acute rejection. For the ABCB1-A gene, only 12 samples were available, which was considered an insufficient sample size to perform an inferential analysis.

Discussion

According to data from the Global Burden of Disease Program in 2019, liver disease ranks eleventh as a cause of global mortality, with 2.4 million deaths during that year [20]. Liver transplantation corresponds to the last therapeutic possibility

Table 1. Sociodemographic aspects and clinical outcomes of the immunosuppressed patients with tacrolimus who participated in the study (n=17).

Variable	Frequency (%)
Sex	
Male	9 (53%)
Female	8 (47%)
Age (years), median (range)	56 (18-68)
Weight (kg), median (range)	71 (53-100)
Child – Pugh Index (score)	10 (5-13)
Índice MELD (puntuación)	21 (9-30)
ICU-LOS (days), median (range)	5 (2-58)
Hospital LOS (days), median (range)	17 (7-58)
Follow up (months), median (range)	12 (1-35)
Indication for transplant	
Alcoholic liver disease	5 (29%)
Primary sclerosing cholangitis	3 (18%)
Secondary biliary cirrhosis	1 (6%)
Cryptogenic cirrhosis	1 (6%)
Primary biliary cholangitis	1 (6%)
Wilson's disease	1 (6%)
Non-alcoholic steatohepatitis	1 (6%)
Acute liver failure	1 (6%)
Autoimmune hepatitis	1 (6%)
Hepatitis C	1 (6%)
NASH vs autoimmune hepatitis	1 (6%)
Post-surgical complications	13 (76%)
Bile	7 (54%)
Vascular	4 (31%)
Infectious	2 (15%)
Perioperative acute kidney injury	4 (24%)
Acute clinical rejection	13 (76%)
Postoperative days after diagnosis of rejection	
Tacrolimus levels during the rejection event or at day 6-10 in patients without rejection	
Infratherapeutic	15 (88%)
Supratherapeutic	1 (6%)
Therapeutic	1 (6%)
Tacrolimus dosage during the rejection event or on day 6-10 in patients without rejection	
Less than 0.04 mg/kg/day	0 (0%)
Between 0.04-0.09 mg/kg/day	10 (59%)
Greater than or equal to 0.1 mg/kg/day	7 (41%)
Pharmacological toxicity (WHO-UMS grades)	
Neurotoxicity	
Possible	5 (29%)
Nefrotoxicity	
Likely	1 (6%)
Possible	2 (12%)
Unlikely	3 (18%)
Hepatotoxicity	
Probable	2 (12%)
Deaths during follow-up	3 (18%)

MELD: Model for End-stage Liver Disease; ICU: Intensive Care Unit; NASH: nonalcoholic steatohepatitis.
Source: Authors' own elaboration.

Table 2. Sociodemographic factors, important clinical outcomes and univariate comparative analysis between the groups called "expressive" and "non-expressive" according to the genotyping of CYP3A5 (rs776746).

Variables	Non-expressive (CYP3A5*3/*3) (n = 9)	Expressive (CYP3A5*1/*1 + CYP3A5*1/*3) (n = 7)	p-value
Age (Mean, standard deviation)	55 (21)	42 (8)	0.61
Females	5 (57%)	4 (44%)	0.53
Child-Pugh (Mean, standard deviation)	9 (2)	10 (2)	0.44
MELD (Mean, standard deviation)	19 (5)	21 (6)	0.33
Tacrolimus levels (Mean, standard deviation)	8 (4)	6 (2)	0.38
Subtherapeutic levels	4 (44%)	5 (57%)	0.89
Perioperative acute kidney injury	3 (33%)	1 (16%)	0.78
Clinical rejection	4 (44%)	7 (100%)	0.05

Source: Authors' own elaboration.

in the management of end-stage chronic liver disease, acute liver failure and some primary liver neoplasms. In Colombia, according to data from the National Institute of Health, 155 liver transplants were performed in 2021, a figure that has been increasing after the SARS-CoV-2 pandemic, when there was a marked decrease in this type of procedures [21,22].

Acute rejection is a major cause of dysfunction, allograft loss, and death in patients undergoing solid organ transplantation. It occurs in 25-46% of patients, with a highest incidence during the first 6-10 days. Immunosuppression regimens not only prevent the appearance of rejection, but are the basis of treatment in those cases in which this event occurs [23]. Tacrolimus is an immunosuppressive drug that was developed as an alternative to the use of cyclosporine due to its better bioavailability [24], with a 15% lower mortality at one year, increased graft survival rates at one year, and drug adherence rates, decreased rates of acute rejection and steroid-resistant rejection when compared with patients undergoing regimens based on cyclosporine [25].

CYP3A5 is the main enzyme in the metabolism of tacrolimus and has hepatic and intestinal representation. P-glycoprotein is a drug efflux transporter at the intestinal level [4]. Polymorphisms

Table 3. Genotype of the included patients.

Genotyping	Frequency (%)
MDR1	
ABCB1-A (rs2032582)	n=12
TT	1 (8.3%)
TG	5 (41.6%)
GG	6 (50%)
ABCB1-B (rs1045642)	n=17
CC	4 (23.5%)
TC	9 (52.9%)
TT	4 (23.5%)
CYP3A5 (rs776746)	n=16
CYP3A5*1/*1	3 (18.7%)
CYP3A5*1/*3	4 (25%)
CYP3A5*3/*3	9 (56.2%)

Source: Authors' own elaboration.

in the genes that encode both proteins have been considered a probable cause of the interindividual pharmacokinetic variability of the drug and have been considered as the basis of possible models of personalized medicine in liver transplantation [8-10]. It is considered that during the first five weeks after transplantation the pharmacokinetics of this drug is mainly influenced by the genotyping of the recipient [26,27].

Table 4. Univariate analysis between the groups called "expressive" and "non-expressive" according to the genotyping of ABCB1-B (rs1045642).

Variables	Expressive (TC - CC) (n = 13)	Non-expressive (TT) (n = 4)	p-value
Age (Mean, standard deviation)	52 (19)	42 (15)	0.36
Female (n, %)	5 (38%)	3 (75%)	0.56
Child-Pugh (Mean, standard deviation)	9 (2)	10 (1)	0.66
MELD (Mean, standard deviation)	19 (6)	21 (3)	0.53
Tacrolimus levels mg/dl (Mean, standard deviation)	8 (4)	5 (2)	0.46
Subtherapeutic levels	7 (54%)	2 (50%)	0.92
Perioperative acute kidney injury	9 (69%)	2 (50%)	0.50
Acute clinical rejection	9 (69%)	3 (75%)	1

MELD: Model for End-stage Liver Disease.
Source: Authors' own elaboration.

This study describes a cohort of liver transplant recipients in Colombia, the frequency of presentation of important polymorphisms in these genes and makes an approximation of their association with outcomes such as pharmacological toxicity and acute rejection. It is known that the CYP3A5 genotype varies according to racial and population factors; approximately 10-20% of Caucasians, 20-80% of Asians, and 55-65% of African Americans express some allele for CYP3A5*1 [5]. In our study population, 18% of patients were homozygous for the CYP3A5 *1/*1 wild-type gene, 25% were heterozygous, and 56% were homozygous for CYP3A5*3; with 43% "expressers", probably as an intermediate population between Caucasian and Asian, similar to what was published by Buendía [28].

It has been observed that patients with the "non-expressive" genotype (CYP3A5*3/*3) require lower doses of the drug and have a higher risk of drug toxicity. Furthermore, patients with the "expressive" genotype have been associated with higher rates of acute rejection [29,30]. The group led by Gómez-Bravo et al. documented a 10.2% incidence of histologically confirmed rejection in a Spanish population, finding that "expressing" patients had rejection rates approximately 1.5 times higher in the first three months compared to with the "non-expressive" [5].

On the other hand, Uesugi and collaborators [27] carried out a study in the Japanese population, in which they discovered that the presence of the CYP3A5*1 allele in the transplanted liver was associated with a higher incidence of acute rejection between days 10 and 23 post-transplant. No differences were found in tacrolimus levels between those who experienced rejection and those who did not.

In our series of patients, an overall acute clinical rejection rate of 76% was found, which is considerably higher than the range of 25-46% reported in the literature [22]. 44% of the "non-expressive" patients had an episode of clinical acute rejection versus 100% of the "expressive" patients, reaching statistical significance in the univariate analysis. However, a rate of subtherapeutic levels of 88% was observed until day 10, and this was due to the fact that 59% of patients were receiving doses less than 0.1 mg/kg/day, in the context of a progressive introduction scheme of calcineurin inhibitors.

Although this raises questions about the initiation of our maintenance immunosuppression, the findings suggest that patients with any wild-type allele have higher rates of clinical acute rejection, which is valuable, biologically plausible, and correlates with findings described elsewhere [5,26].

All patients who experienced clinical rejection in our study improved their biochemical parameters by adjusting immunosuppression, and no cases of rejection-related retransplantation were reported during follow-up.

The presence of adverse events secondary to the administration of tacrolimus has been studied in relation to CYP3A45 genotyping. Supratherapeutic levels of tacrolimus have been associated with the development of nephrotoxicity [4]. Coller et al. [11] evaluated as a secondary outcome in their study, the development of nephrotoxicity in relation to the presence of CYP3A5 polymorphisms in 29 patients. Renal toxicity occurred in 14% of patients, with no statistically significant difference between the groups. Similarly, three patients with nephrotoxicity (17%) were identified in our study. No statistically significant difference was found between "expressive" and "non-expressive" with respect to this or other types of toxicity in our study, nor in the rate of perioperative acute kidney injury.

Regarding the polymorphisms of the ABCB1 gene, it has been described that the presence of the homozygous genotype for both mutations is approximately 32% of Caucasians, 62% of Americans of European ancestry, 27% of Americans of Asian ancestry, and 35 % of Americans with Mexican ancestry [9]. In our cohort, 50% were homozygous for GG in ABCB1-A and 23% were homozygous for the mutated TT allele in ABCB1-B.

It is known that these mutations generate a decrease in the expression of intestinal P-glycoprotein and their impact on the pharmacokinetics of tacrolimus is better described in patients with kidney transplants, in whom the presence of the mutated allele is associated with lower C0/dose rates mainly in ABCB1-B [31]. However, its impact on the pharmacokinetics of tacrolimus in liver transplant recipients is still a topic of discussion. Provenzani et al. [9] found no differences in drug doses or incidence of graft rejection in patients undergoing liver transplantation according to ABCB1 genotyping. In our study we did not find statistically significant differences in the presence of clinical acute rejection or in secondary outcomes according to MDR-1 genotyping.

Limitations of this study include the low number of patients included, because the study period coincided with the pandemic, the death of eight patients who did not receive immunosuppression with tacrolimus, and the quality of the specimen in some samples did not allow the genetic study. The high rates of infratherapeutic levels associated with acute clinical rejection events may be a confounding factor, which is why efficient optimization of drug levels in the early post-transplant period is suggested. The formulation of multicenter studies is recommended that allow a broader knowledge of the population distribution of these genes and are the basis for proposing personalized immunosuppression schemes according to the genotyping.

Conclusions

This study describes the frequency of single nucleotide polymorphisms in key proteins for tacrolimus metabolism in liver transplant recipients. The presence of the "expressive" genotype was associated with higher rates of acute clinical rejection, with the vast majority of events documented in the first 14 days post-transplant. No association was found with adverse drug events or other events such as perioperative acute kidney injury. No differences were found in clinical acute rejection and toxicity in the different MDR-1 polymorphisms.

Compliance with ethical standards

Informed consent: The patients signed the informed consent for the data collection. All protocols conformed to the ethical guidelines of the Declaration of Helsinki of 1975 and the guidelines of Resolution 008430 of 1993 of the Ministry of Health of Colombia. This study was approved by the ethics committee of the Hospital Universitario San Vicente Fundación.

Conflict of interest: The authors declare no conflicts of interest.

Use of artificial intelligence: The authors declared that they did not use artificial intelligence (AI)-assisted technologies (such as large language models, chatbots, or image creators) in the production of this work.

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Author's contributions

- Conception and design of the study: Lina María Botero-Mora, Erika Fernanda Lindarte-Rincón, Jefferson Antonio Buendía, Luis Guillermo Toro-Rendón.
- Acquisition of data: Lina María Botero-Mora, Jefferson Antonio Buendía, Luis Guillermo Toro-Rendón.
- Data analysis and interpretation: Lina María Botero-Mora, Erika Fernanda Lindarte-Rincón, Jaime Alberto Ramírez-Arbeláez, Jefferson Antonio Buendía.
- Drafting the manuscript: Lina María Botero-Mora, Luis Manuel Barrera-Lozano, Jaime Alberto Ramírez-Arbeláez.
- Critical review: Lina María Botero-Mora, Erika Fernanda Lindarte-Rincón, Luis Manuel Barrera-Lozano, Jaime Alberto Ramírez-Arbeláez, Luis Guillermo Toro-Rendón.

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