The Prognostic Role of CYP Enzyme in Kidney Transplantation: A Single Centre Experience

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ABSTRACT

Background. The main goal of immunosuppressive agents is to reach a balance of preserving allograft function while minimizing adverse effects. The purpose of our research is to corroborate the role of CYP3A enzyme in developing individual medication therapy via measuring medicine levels in patients' blood samples.

Methods. This retrospective analysis studies 15 kidney transplant recipients. We carried out genotyping (*CYP3A5, CYP3A4*) after isolating DNA and RNA in patient and donor blood samples; we also determined CYP3A4 messenger RNA expression in case of recipients. Tacrolimus blood levels, dosage, and tacrolimus concentration normalized by dose and the body weight (C_0/D ratio) were evaluated.

Results. In this research, recipients were divided into 2 groups based on their *CYP3A5* genotype. Those who carry *CYP3A5*1* allele (**1/*1* or **1/*3*) are CYP3A5 expressors, whereas those who are homozygous for the nonfunctional *CYP3A5*3* allele are CYP3A5 nonexpressors. There were 3 patients with functioning CYP3A5 enzyme (patients with *CYP3A5*1/*3* genotype) where increased tacrolimus metabolism was expected. Our data show that C_0/D ratio of CYP3A5 nonexpressors was around 3 times higher than of CYP3A5 expressors.
Looking at CYP3A4 enzyme, we found 1 patient carried *CYP3A4*22/*22* genotype where we expected decreased CYP3A4 expression. It is clear that this patient had adequate therapy medication levels (9.50 μg/L) despite having received very low dosage of tacrolimus (0.03 mg/weight/d).

Conclusions. Our results confirmed the importance of determining CYP status of recipients after a transplant because individual differences were observed in tacrolimus treatment that were partly influenced by CYP status of recipients.

SIGNIFICANT developments in kidney transplant have been achieved through advances in immunosuppressive therapies over the last decades [1]. Chronic allograft nephropathy, which is partly related to calcineurin inhibitor toxicity, is still a relevant problem post kidney transplant and adds to poor long-term allograft survival, in spite of the improvement in modern immunosuppression and subsequent developments in short-term graft survival. The main goal of immunosuppressive agents is to reach a balance of preserving allograft function while minimizing adverse effects [2].

Tacrolimus is a T cell proliferation inhibitor that is generally used in kidney transplant. After transplant, it is part of the maintenance immunosuppressive therapy in combination with mycophenolic acid and corticosteroids [3]. Tacrolimus therapy requires frequent serum trough level (C_0) monitoring and dose adjustment because of its narrow therapeutic index.

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Overtreatment and undertreatment may have harmful effects on mortality and graft survival, resulting in increased risk of cardiovascular diseases, malignant neoplasms, rejection, and post-transplant diabetes mellitus [4].

Age, drug interactions, body weight, and genetic background of enzymes that play a part in tacrolimus metabolism (cytochrome P450 3A4-CYP3A4 and cytochrome P450 3A5-CYP3A5 in particular) as predictive factors may help in dose optimization of tacrolimus [5]. The dominating enzymes that play a part in tacrolimus metabolism are CYP3A5 and CYP3A4 and are expressed mainly in the liver, intestine, and kidney [6,7].

The presence of *CYP3A4* and *CYP3A5* genetic variants significantly affects tacrolimus clearance and dosage. *CYP3A5*3* is a loss-of-function variant that causes no CYP3A5 enzyme production [6]. Allele carriers of *CYP3A5*1* (*CYP3A5*1/*1* or *CYP3A5 *1/*3*, CYP3A5 expressor) produce high levels of functional CYP3A5 opposed to homozygous carriers of *CYP3A5*3* (CYP3A5 nonexpressor). Tacrolimus metabolism is faster in recipients carrying *CYP3A5*1* allele than CYP3A5 nonexpressors. CYP3A5 expressors might require higher doses to achieve target blood concentrations. Relative contribution of CYP3A5 to tacrolimus metabolism is significantly higher than CYP3A4 [7,8].

*CYP3A4*1B* and *CYP3A4*22*, which are genetic variants of *CYP3A4*, need to be taken into account during prediction of tacrolimus-metabolizing capacity as well. *CYP3A4*1B* appears to contribute to increased transcription of *CYP3A4* gene [8]. *CYP3A4*22* is a variant that has been proposed to be in connection with reduced production of functional CYP3A4 protein [6,9]. The White population widely carry these variant alleles but are rarely homozygous for both *CYP3A5*3* and *CYP3A4*22* [6].

*CYP3A4*22* leads to lower messenger RNA (mRNA) expression and reduced CYP3A4 enzyme activity. At the same time *CYP3A4*22* is suggested to be evaluated together with the *CYP3A5* genotype [10,11].

MATERIALS AND METHODS

Our research is conducted at the Department of Transplantation of University of Debrecen Hungary, in association with the Institute of Enzymology, Research Center for Natural Sciences. The research involves patients who received kidney transplant in 2021.

We carried out genotyping (*CYP3A5, CYP3A4*) (n = 15) after isolating DNA and RNA in patient and donor blood samples; we also determined CYP3A4 mRNA expression (n = 13) in case of recipients (in leukocytes isolated from peripheral blood). Blood samples were taken no more than 2 weeks after the transplant.

Measurements were done based on CYPtest diagnostic system in the Enzymology Institute [11,12]. We used retrospective clinical data processing. The research involves kidney transplant recipients who are older than 18 years.

The main purpose of our research is to corroborate the role of CYP3A enzyme in developing individual medication therapy via measuring medicine levels in patients' blood samples. Tacrolimus blood level is measured daily in the early postoperative period; later it is measured weekly and monthly. We present our results (up until now) based on monthly blood levels. We are looking for connections between the CYP3A status of patients and their tacrolimus blood level. Because the tacrolimus metabolizing ability of the transplanted kidney can locally affect graft operation, we are determining the *CYP3A5* genotype of donors from blood or tissue samples.

RESULTS

This retrospective analysis studies 15 kidney transplant recipients. All 15 recipients received kidney from deceased brain-dead donors. Causes of brain death of donors were stroke (73%), trauma (20%), and other (7%). A total of 27% of transplanted kidneys were from expanded criteria donors, and 73% were from standard criteria donors. The average (SD) age of recipients was 54 (14.52) years. Among examined recipients end-stage kidney disease was caused by polycystic kidney disease, diabetes nephropathy, IgA nephropathy, etc. Diabetes mellitus was diagnosed in 5 recipients (33%). One month after transplant, recipients had good graft function (mean [SD] serum creatinine, 105 [27] mmol/L) (Table 1).

Patients received tacrolimus (once-daily tacrolimus extended-release or twice-daily immediate-release agents) for basic immunosuppression, combined with corticosteroids and mycophenolate mofetil. In line with local protocol, dosage of first phase calcineurin inhibitor therapy was 0.1 mg/weight/d. Target value of tacrolimus blood level was 10 to 13 ng/mL (12 hours after administering the last calcineurin inhibitor agent).

We would like to involve more patients and examine them separately based on if they received once-daily tacrolimus extended-release or twice-daily immediate-release formulation. They were treated as a single group at this point because of the small sample count. To be able to compare, data were converted from once-daily extended-release tacrolimus dosage to twice-

Table 1. Demographic and Clinical Data of Recipients

Demographic and Clinical Data of Recipients	Value
Age at time of transplant, mean (SD), y	54 (14.5)
Sex, % female/male	33/67
Body weight at time of transplant, mean, kg	68
Patient with diabetes mellitus, %	33
Cold ischemia time, mean, h	15.3
ECD graft/SCD graft, %	27/73
Maintenance therapy (tacrolimus [Prograf/Envarsus]), %	60/40
Serum creatinine at 1 mo, mean (SD), mmol/L	105 (27)
Primary cause of ESKD, No.	
Polycystic kidney disease	3
IgA nephropathy	2
Diabetic nephropathy	3
Lupus erythematosus	1
Alport syndrome	1
Dense deposit disease	1
Toxic nephropathy	2
Unspecified contracted kidney	1
Chronic kidney failure - etiology uncertain	1

ECD, expanded criteria donor; ESKD, end-stage kidney disease; SCD, standard criteria donor.

Table 2. Tacrolimus Blood Concentration, Doses, and Tacrolimus Concentration Normalized by Dose and Body Weight in Kidney Transplant Recipients by CYP3A5 Genotype, 1 Month After Kidney Transplant

CYP3A5	No.	Tacrolimus, mean (SD), dose/weight/d	Blood Concentration of Tacrolimus, mean (SD), μg/L	Tacrolimus Concentration Normalized by Dose and Body Weight, mean (SD)
*CYP3A5 *3/*3*	12	0.089 (0.038)	10.68ug/L (2.52)	140.36 (66.07)
*CYP3A5 *1/*3*	3	0.204 (0.074)	7.83ug/L (0.49)	42.12 (1570)
*CYP3A5 *1/*1*	0	NA	NA	NA

NA, not applicable.

daily immediate-release tacrolimus dosage during our calculations (0.7 to 1, suggested by European Medicines Agency (EMA) [13]).

In this research, recipients were divided into 2 groups based on their *CYP3A5* genotype. Those who carry *CYP3A5*1* allele (**1/*1* or **1/*3*) are CYP3A5 expressors, whereas those who are homozygous for the nonfunctional *CYP3A5*3* allele are CYP3A5 nonexpressors. Tacrolimus blood levels, dosage, and tacrolimus concentration normalized by dose and the body weight (C_0/D ratio) were evaluated for both groups.

There were 3 patients with functioning CYP3A5 enzyme (patients with *CYP3A5*1/*3* genotype) where increased tacrolimus metabolism was expected. During the follow-up after 1 month, we observed that the 3 patients had received high volume of tacrolimus (mean [SD], 0.204 [0.07] mg/weight/d). At the same time their tacrolimus blood level (7.83 [0.49] μg/L) was lower than expected. In case of CYP3A5 nonexpressors, average tacrolimus dose (0.089 [0.038] mg/weight/d) was lower with adequate tacrolimus blood level (10.68 [2.52] μg/L). CYP3A5 expressors had lower C_0/D ratio. (42.12 [15.70] vs 140.36 [66.07]) (Table 2).

Looking at CYP3A4 enzyme, we found 2 patients carried *CYP3A4*1B/*22* genotype, but in one case CYP3A5 enzyme was functional, so the latter plays a part in tacrolimus metabolism. In this group the average tacrolimus dose was 0.17 (0.15) mg/weight/d, while patients (n = 12) who carried *CYP3A4*1/*1* genotype received average tacrolimus dose of 0.11 (0.045) mg/weight/d. C_0/D ratio was almost the same in the 2 groups.

One patient carried *CYP3A4*22/*22* genotype, where we expected decreased CYP3A4 expression, according to literature. It is clear that this patient had adequate therapy medication levels (9.50 μg/L) despite having received very low dosage of tacrolimus (0.03 mg/weight/d). This also means that their C_0/D ratio was high (297.04) (Table 3).

Regarding CYP3A4 mRNA expression, the CYP3A5 nonexpressor recipients (n = 12) were divided into 3 groups: poor, intermediate, and extensive CYP3A4 metabolizers. Four recipients were intermediate, and 7 recipients were poor CYP3A4 metabolizers. We did not identify an extensive CYP3A4 metabolizer. Until now, our results have not confirmed that patients with low CPY3A4 mRNA expression required decreased dosage of tacrolimus to reach adequate therapy medication levels because C_0/D ratio was almost the same for poor and intermediate CYP3A4 metabolizers (Table 4).

We also determined the CYP3A genotype of donors, during which we observed that only 2 donors were part of the group that expresses CPY3A5 enzyme. One recipient, who had received the kidney of mentioned donors, received higher dosage of tacrolimus (0.183 mg/weight/d) to reach adequate therapy medication levels (9.7ug/L). This recipient was CYP3A5 nonexpressor and also exhibited reduced mRNA CYP3A4 expression. Looking at *CYP3A4* genotype of donors, we found

Table 3. Tacrolimus Blood Concentration, Doses, and Tacrolimus Concentration Normalized by Dose and Body Weight in Kidney Transplant Recipients by CYP3A4 Genotype, 1 Month After Kidney Transplant

CYP3A4	No.	Tacrolimus, mean, dose/weight/d	Blood Concentration of Tacrolimus, mean SD), μg/L	Tacrolimus Concentration Normalized by Dose and Body Weight, mean (SD)
*CYP3A4 *1/*1*	12	0.11 (0.045)	10.36 (2.74)	109.90 (50.76)
*CYP3A4 *22/*22*	1	0.03	9.50	297.04
*CYP3A4 *1B/*22*	2	0.17 (0.15)	8.95 (1.90)	97.46 (98.87)

Table 4. Tacrolimus Blood Concentration, Doses, and Tacrolimus Concentration Normalized by Dose and Body Weight in Kidney Transplant Recipients by CYP3A4 mRNA Expression, 1 Month After Kidney Transplant

CYP3A4 mRNA expression	No.	Tacrolimus, mean (SD), dose/weight/d	Blood Concentration of Tacrolimus, mean (SD), μg/L	Tacrolimus Concentration Normalized by Dose and Body Weight, mean (SD)
Intermediate metabolizers	4	0.083 (0.028)	11.60 (3.03)	148.97 (49.61)
Poor metabolizers	7	0.093 (0.047)	9.79 (2.10)	134.55 (81.73)
Extensive metabolizers	0	NA	NA	NA

mRNA, messenger RNA; NA, not applicable.

2 of them carried *CYP3A4*1/*1B* genotype, and the others (n = 13) carried *CYP3A4*1/*1* genotype.

DISCUSSION

Because long-term graft survival is largely affected by immunosuppressive therapy, the above research is instrumental in life expectancy of patients.

After a kidney transplant, the largest challenge for clinical specialist is long-term maintenance of kidney grafts. Immunosuppressive therapy dosage plays a big part in this maintenance. Although tacrolimus is one of the most commonly used immunosuppressive therapies, its administration can be the cause for graft rejection and numerous adverse effects [14]. Calcineurin inhibitors are direct nephrotoxins, and while chronic allograft nephrotoxicity is still an issue, they show significantly lower acute rejection rates [1].

Characteristics of pharmacokinetics of tacrolimus is defined by individual diversity in system clearance and first-pass metabolism. The main factor in these differences is *CYP3A5* polymorphisms and their respective effect on tacrolimus metabolism [1].

To avoid potentially harmful overexposure or underexposure, personalized medicine is targeting to adjust prescriptions based on information of genetic polymorphisms. To provide description of genotype-phenotype association and therefore to improve long-term kidney transplant outcomes, various genetic polymorphism studies have been carried out [15]. Significant associations have been reported between CYP3A5 and the doses required for kidney transplant recipients [1].

Our data show that C_0/D ratio of CYP3A5 nonexpressors was around 3 times higher than of CYP3A5 expressors. According to Rojas et al CYP3A5 nonexpressors produced C_0/D ratio between 1.8 and 2.5 times higher than CYP3A5 expressors in the first, post-transplant year, indicating that recipient genotypes take part in rejection or toxicity. Additionally, expressors do not exhibit a risk of acute nephrotoxicity and likely have a greater risk of tacrolimus-related chronic nephrotoxicity and acute rejection. Physicians familiarizing with *CYP3A5* genotype could help them in determining tacrolimus doses so they can achieve more effective dosage and identify actions to reduce the risk of nephrotoxicity and rejection [7].

Our research shows that our one patient who carried *CYP3A4*22/*22* genotype achieved adequate therapy medication levels despite having received very low dosage of tacrolimus. According to Pallet et al, among CYP3A5 nonexpressors, who may require lower tacrolimus doses than those that carry one CYP3A51 allele, a subgroup (carrying *CYP3A4*22*) may still requisite lower tacrolimus doses to achieve the target tacrolimus concentration [16].

The outcomes may help to get rid of fluctuations in tacrolimus levels and improve tolerability of tacrolimus in recipients and may be important when determining the initial and maintenance tacrolimus doses [17]. Patients at high risk of tacrolimus-related complications may be identified before their kidney transplant by putting these findings in use regarding liver metabolism pharmacogenetics and using this routine parameter (C_0/D) [7].

Our research has a number of limitations. First, it is a single-center experience with low sample count and short duration of follow-ups. Furthermore, we did not take multidrug immunosuppressive regimens into account, which that may have varied among patients (secondary immunosuppressant and/or corticosteroid).

CONCLUSIONS

Our results have confirmed the importance of determining CYP status of recipients after transplant because individual differences were observed in tacrolimus treatment, which were partly influenced by CYP status of recipients.

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