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The relationship between tacrolimus concentration-dose ratio and genetic polymorphism in patients subjected to renal transplantation

Povezanost odnosa koncentracija-doza takrolimusa i genetskog polimorfizma kod bolesnika sa transplantiranim bubregom

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Abstract

Background/Aim. Tacrolimus concentration-dose ratio as a potential therapeutic drug monitoring strategy was suggested to be used for the patients subjected to renal transplantation. The aim of this study was examining the relationship between tacrolimus concentration-dose ratio, suggested to be used as a therapeutic drug monitoring strategy and the polymorphisms of genes encoding the most important enzymes, such as CYP3A5 and CYP3A4, as well as the transporter P-glycoprotein, for its metabolism and elimination. **Methods.** The study was designed as a prospective case series study, in which the unit of monitoring was the outpatient examination of 54 patients subjected to renal transplantation. Genotyping was performed by 7500 Real-Time PCR System by assessing allelic discrimination based on TaqMan® methodology. **Results.** Patients (n = 13) who were treated with less than 2 mg of tacrolimus/day (0.024 ± 0.006 mg/kg/day) had the tacrolimus concentration-dose ratio larger than 150 ng/mL/mg/kg. In this group, 84.62% patients had CYP3A5 *3*3 allele. All of these patients had CYP3A4 *1*1/*1*1B allele. Regarding ABCB1 C3435T gene, 30.77% of patients had the TT gene variant, while 69.23% of our patients had CC and CT gene variants. **Conclusion.** Tacrolimus concentration-dose ratio greater than 150 ng/mL/mg/kg is cut-off value in patients subjected to renal transplantation which might point to patients who are poor CYP3A5 metabolizers and/or with dysfunctional P-glycoprotein.

Key words:
kidney transplantation; tacrolimus; dose-response relationship, drug; polymorphism, genetic.

Apstrakt

Uvod/Cilj. Odnos koncentracija-doza takrolimusa, kao potencijalna strategija terapijskog monitoringa lekova, upućuje na to da se može koristiti kod bolesnika sa transplantiranim bubregom. Cilj ove studije je bio da ispita vezu između odnosa koncentracija-doza takrolimusa koji je sugerisan kao strategija terapijskog monitoring lekova i genskog polimorfizma gena koji kodiraju najznačajnije enzime, CYP3A5 i CYP3A4, kao i transporter P-glikoprotein, za metabolizam i eliminaciju takrolimusa. **Metode.** Studija je osmišljena kao prospektivna serija slučajeva, u kojoj je jedinica monitoringa bio ambulantni pregled 54 bolesnika sa transplantiranim bubregom. Genotipizacija je urađena na aparatu 7500 Real-Time PCR System za procenu za diskriminacije alela koja se bazira na TaqMan® metodologiji. **Rezultati.** Bolesnici (n = 13) koji su lečeni sa manje od 2 mg takrolimusa na dan (0,024 ± 0,006 mg/kg/dan) imali su odnos koncentracija-doza takrolimusa veći od 150 ng/mL/mg/kg. U ovoj grupi, 84,62% bolesnika je imalo CYP3A5 *3*3 alele. Svi ovi bolesnici su imali CYP3A4 *1*1/*1*1B alele. Što se tiče ABCB1 C3435T gena, 30,77% bolesnika je imalo TT gensku varijantu, dok je 69,23% njih imalo CC i CT gensku varijantu. **Zaključak.** Odnos koncentracija-doza takrolimusa veći od 150 ng/mL/mg/kg je granična vrednost kod bolesnika sa transplantiranim bubregom koji može da ukaže na one bolesnike koji su spori CYP3A5 metabolizeri i/ili su sa disfunkcionalnim P-glikoproteinom.

Ključne reči:
transplantacija bubrega; takrolimus; lekovi, odnos doza-reakcija; polimorfizam, genetički.

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Introduction

Tacrolimus is one of the most important immunosuppressive drugs used for renal transplantation [1]. It is a "critical dose" drug because of its narrow therapeutic range. Underexposure to tacrolimus may result in an acute rejection and graft dysfunction, while overexposure might be followed by serious adverse effects [2]. The clinical usage of tacrolimus can be complicated due to significant inter-individual and intra-individual variability of this drug, as well as significant differences in bioavailability [3]. It is well known from clinical practice that patients who are treated with the equal doses of this drug could have high variability of tacrolimus blood concentrations.

Numerous factors have been identified as contributors to the high tacrolimus pharmacokinetic variability: age, gender, body mass index, albumin concentration, liver dysfunction, hematocrit, time elapsed after transplantation, hepatitis C status, diabetes status, diarrhoea, corticosteroid dosage, drug-drug interactions and food administration [3–6]. As a result, therapeutic drug monitoring (TDM) is particularly important. Recently, tacrolimus concentration-dose ratio (C/D ratio), as a potential TDM and target concentration intervention (TCI) strategy, has been suggested to be used for the patients subjected to renal transplantation [5, 6–8]. The tacrolimus C/D ratio is the ratio between tacrolimus trough concentrations (TTC) (ng/mL) and 24h dose normalized by patient's weight (mg/kg/day) [5]. Tacrolimus C/D ratio, together with TTC, would provide a better estimation of the influence of additional factors, like gender and comedication on tacrolimus exposure in these patients.

Genetic polymorphism is also considered to be one of the most significant causes of tacrolimus pharmacokinetic variability [5, 9–11]. Since polymorphic cytochrome P450 isoenzyme family (CYP) is the most important system involved in tacrolimus biotransformation and elimination, genotyping CYP polymorphisms provides important information that can predict tacrolimus exposure in patients subjected to renal transplantation [9]. Tacrolimus is metabolized mainly by CYP3A4 and CYP3A5 isoenzymes [9]. It is also a substrate of P-glycoprotein efflux pump. P-glycoprotein lowers the blood concentration of tacrolimus by pumping the absorbed tacrolimus back into the intestinal lumen [12]. Polymorphisms of genes which encode these isoenzymes and efflux pump can have a significant influence on tacrolimus blood concentrations in these patients [9, 12].

The aim of this study was to examine the relationship between tacrolimus C/D ratio and polymorphisms of genes encoding the most important enzymes, CYP3A5 and CYP3A4 and transporter, P-glycoprotein, for its metabolism and elimination in order to estimate the influence of genetic polymorphisms on tacrolimus exposure in patients subjected to renal transplantation.

Methods

Study design

The study was designed as a prospective case series study. The unit of monitoring was outpatient examination of 54 patients subjected to renal transplantation in the Centre for Solid Organ Transplantation in the tertiary health care university hospital, the Military Medical Academy (MMA), Belgrade, Serbia. They were all monitored during 4 years, from September 2010 to January 2015, starting one month after renal transplantation.

Patients and therapeutic protocol

All patients were treated in accordance with the established therapeutic protocol in the Centre, as described in the earlier studies [7, 13]. After kidney transplantation, they were subjected to the triple-drug-therapy, including corticosteroids (methylprednisolone or prednisone), myco-phenolate mofetil and tacrolimus (Prograf®, Astellas, Japan), with or without the addition of an induction agent (anti-T lymphocyte globulin) in the early phase after the transplantation. The other drugs were administered according to comorbidity.

On the day of transplantation, tacrolimus was introduced in an initial oral dose of 0.1–0.3 mg/kg/day, divided into 12-hour intervals. The patients were given a dose of 500 mg of methylprednisolone, intravenously, on the day of the surgical intervention, before the transplantation itself; the next 2 days the dose was 250 mg/day, and then, it was reduced to 125 mg/day in the following 2 days, followed by 3 days with a dose of 1.5 mg/kg/day. During the second week after transplantation, a dose of 0.3 mg/kg/day of prednisone was administered orally; the same dosage was used until the end of the first month. The prednisone dose of 10 mg/day was prescribed until the end of the first year after transplantation, while 10 mg dose was recommended every other day, during the second year of treatment and later on. Mycophenolate mofetil was given orally, 1 g, twice a day, starting 2 days before the kidney transplantation. Three months after transplantation, mycophenolate mofetil dose was reduced to 500 mg, twice a day. After this dose reduction, mycophenolate mofetil was taken permanently. Anti-thymocyte globulin was administered intravenously (as a slow intravenous infusion) as a series of divided doses during the first post-transplant week (in a dose of 2–4 mg/kg/day).

The other drugs were administered according to comorbidity. In order to control hypertension, calcium channel blockers (nifedipine, amlodipine), β adrenergic antagonists (propranolol, carvedilol, bisoprolol, atenolol, metoprolol, nebivolol) and/or diuretics (furosemide) were given. As a prophylaxis for peptic ulcers and surgical stress-related bleeding, H_2-antagonists (ranitidine) or proton pump inhibitors (pantoprazole, esomeprazole) were administered. The doses of all concomitant drugs were always within recommended therapeutic range. All the patients were also treated with cotrimoxazole (for *Pneumocystis Jirovecii* prophylaxis) for 6 post-transplant months.

Therapeutic drug monitoring

Tacrolimus TDM was needed to optimize the dosage regime in patients after renal transplantations. Tacrolimus trough concentrations (TTC) were measured by chemiluminescence microparticles immunoassay (CMIA), ARCHITECT i1000SR

Abbott Laboratories; Abbott Park, Illinois, USA) in the Institute for Medical Research, Department for Clinical and Experimental Immunology, the MMA, Belgrade, Serbia. The whole blood samples were taken 12 h after the evening dose, i.e. 10 min before the morning dose, starting a month after renal transplantation. The recommended target concentration range for TTC has been from 6 to 10 ng/mL. Tacrolimus trough concentrations were measured every other day during 2 weeks after renal transplantation and later, on each control examination. The control examinations were conducted twice a week during the first 3 months after transplantation, once a week for the next 3 months, twice a month from the sixth to the ninth month after transplantation, once a month until the end of the second year, and later on, once in every 3 months.

Genotyping for CYP3A5, CYP3A4 and ABCB1

One blood sample from antecubital vein in a vacutainer with anticoagulant EDTA was taken from each patient. DNA was extracted and isoforms of the enzymes CYP3A5 and CYP3A4 as well as of the transporter ABCB1, were genotyped. The 13 adult patients were genotyped for single nucleotide polymorphism (SNP) of CYP3A5 at position 6986A > G (the *3 or *1, rs776746), CYP3A4 at position -392A > G (the *1 or *1B, rs2740574) and ABCB1 at exon 26 (3435C>T, rs1045642). The genotyping was detected by TaqMan® SNP genotyping assays (Life Technologies, USA) on a 7500 Real-Time polymerase chain reaction (PCR) System (Applied Biosystems, USA).

For CYP3A4, ABCB1 and CYP3A5, the observed genotype (allele) frequencies were in Hardy-Weinberg equilibrium ($p > 0.05$).

Statistical analysis

The complete statistical analysis of data was done using the statistical software package, PASW Statistics 18® [SPSS (Hong Kong) Ltd., Hong Kong]. All variables were presented as frequency of certain categories. Continuous variables were presented as means and standard deviations. Continuous variables were compared by using Mann-Whitney U test. The normality of the data was assessed by using Kolmogorov-Smirnov test. Ratios between tacrolimus daily dose per body weight, TTC and tacrolimus C/D ratio were tested by Spearman's coefficient correlation. All the analyses were estimated at $p < 0.05$ level of the statistical significance.

Ethical approval

The principles of ICH Good Clinical Practice were strictly followed and ethical approval N° 01/31-01-13 from the Ethics Committee of the MMA was obtained for the study protocol N° 910-1.

Results

The most important demographic characteristics and biochemical analyses of renal transplant patients are presented in Table 1. The total of 54 patients was subjected to kidney transplantation (34 males or 63% and 20 females or 37%); the average age was 40.46 ± 11.38. The average body mass index was 21.49 ± 3.18 kg/m^2. The total number of 1,872 outpatient examinations were performed during this follow-up (34.67 ± 10.96 outpatient examinations per patient).

A weak correlation between tacrolimus daily dose per body weight and TTC was shown ($r = 0.233$, $p < 0.001$), while the correlation between tacrolimus daily dose per body weight and its C/D ratio was very strong ($r = -0.859$; $p < 0.001$), (Figures 1 and 2). It was observed that the patients who had tacrolimus C/D ratio larger than 150 ng/mL/mg/kg were treated with less than 2 mg of tacrolimus/day and vice versa (Figure 2).

Calculations were made according to tacrolimus daily dose and it could be concluded that in the patients who were treated with less than 2 mg of tacrolimus/day (0.024 ± 0.006 mg/kg/day) the average TTC was significantly lower (5.82 ± 1.92 ng/mL), and tacrolimus C/D ratio was significantly higher (252.82 ± 101.19 ng/mL/mg/kg) in comparison to those treated with more than 2 mg of tacrolimus/day (0.089 ± 0.041 mg/kg/day), (Table 2). In the patients whose tacrolimus C/D ratio was larger than 150 ng/mL/mg/kg, genotyping of genes encoding the most important enzymes, such as CYP3A5 A6986G and CYP3A4 -A392G, and transporter, ABCB1 C3435T, for tacrolimus metabolism and elimination

Table 1

The most important demographic characteristics and biochemical analyses of renal transplant patients

Demographic characteristics	Values
Total number of patients, n	54
Gender: male/female, n	34/20
Age (years), $\bar{x}$ ± SD	40.46 ± 11.38
Height (m), $\bar{x}$ ± SD	1.74 ± 0.09
Weight (kg), $\bar{x}$ ± SD	67.96 ± 13.47
Body mass index (kg/m^2), $\bar{x}$ ± SD	21.49 ± 3.18
Biochemical analyses	
Haematocrit (vol %), $\bar{x}$ ± SD	0.39 ± 0.05
Blood urea nitrogen (mmol/L), $\bar{x}$ ± SD	10.03 ± 15.29
Creatinine (μmol/L), $\bar{x}$ ± SD	133.64 ± 54.49
Proteinuria (g/24 h), $\bar{x}$ ± SD	0.30 ± 0.29

$\bar{x}$ – mean; SD – standard deviation.

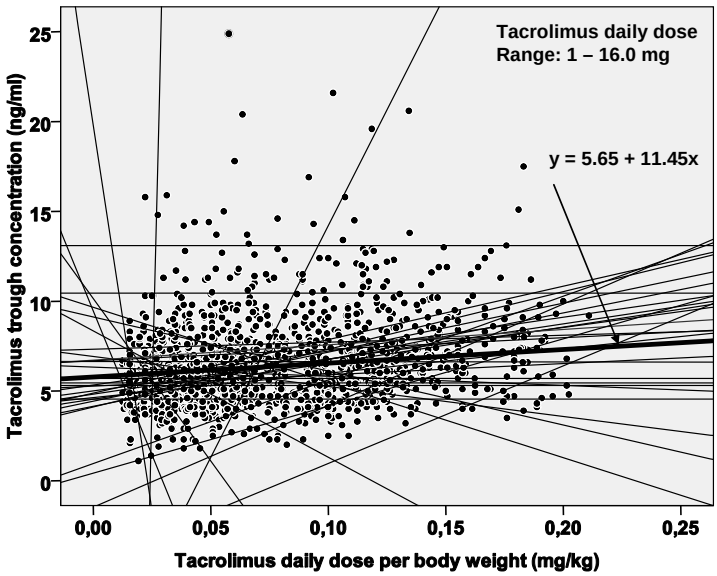

Fig. 1 – Relationship between tacrolimus daily dose and tacrolimus through concentration in patients subjected to renal transplantation

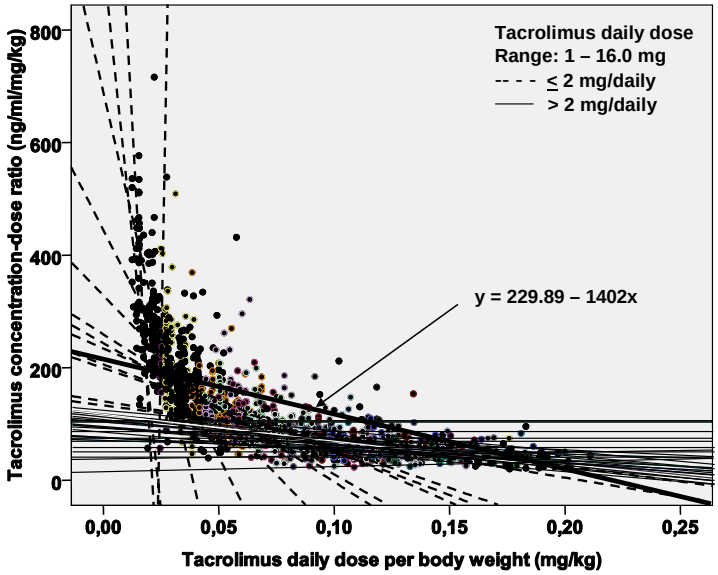

Fig. 2 – Relationship between tacrolimus daily dose and tacrolimus concentration-dose ratio in patients subjected to renal transplantation.

Table 2

Parameters of therapeutic drug monitoring depending on tacrolimus daily dose in patients subjected to renal transplantation

Parameters	Mean ± standard deviation		*p* value
	≤ 2 mg	> 2 mg	(Mann-Whitney test)
Tacrolimus daily dose (mg)	1.60 ± 0.33	5.94 ± 2.91	< 0.001
Tacrolimus daily dose per body weight (mg/kg)	0.024 ± 0.006	0.089 ± 0.041	< 0.001
Body weight (kg)	66.73 ± 8.31	68.16 ± 13.44	0.416
Tacrolimus through concentrations (ng/mL)	5.82 ± 1.92	6.70 ± 2.44	< 0.001
Tacrolimus concentration-dose ratio (ng/mL/mg/kg)	252.82 ± 101.19	92.56 ± 55.01	< 0.001

in renal transplant recipients was performed. The total number of these patients was 13 (Table 3).

In the group of 13 renal transplant recipients most of them (84.62%) had CYP3A5 *3*3 allele (Table 3). On the other hand, 15.38% of patients are homo or heterozygous for CYP3A5 *1 (total of 7.69% *1*1 and 7.69% *1*3). All these patients had CYP3A4 *1*1/*1*1B allele. Regarding ABCB1 C3435T gene, 30.77% of patients had TT gene variant, while CC and CT gene variants were present in 69.23% of them.

Table 3

Genetic polymorphism for CYP3A5, CYP3A4 and ABCB1 C3435T in patients with tacrolimus concentration-dose ratio larger than 150 ng/mL/mg/kg

Genetic polymorphism	% (number of patients)		
CYP3A5 A6986G	AA 7.69 (1)	AG 7.69 (1)	GG 84.62 (11)
CYP3A4 -A392G	AA 92.31 (12)	AG 7.69 (1)	GG -
ABCB1 C3435T	CC 38.46 (5)	CT 30.77 (4)	TT 30.77 (4)

Discussion

A very strong correlation between tacrolimus daily dose expressed per body weight and tacrolimus C/D ratio was found. It was also found a weak correlation between tacrolimus daily dose per body weight and TTC. Tacrolimus trough concentrations, most often used for TDM, are widely accepted as a guide for TCI and individualizing tacrolimus dose requirements in patients subjected to kidney transplantation [14, 15]. On the other hand, although full dose interval area under the concentration-time curve is generally considered as the best marker for tacrolimus exposure, due to its complexity it has not been widely used as a routine method in clinical settings [16, 17]. Quite recently, however, tacrolimus C/D ratio has been suggested as a potentially useful TDM strategy [7]. It, concomitantly with TTC, enabled better estimation of the influence of gender and comedication on tacrolimus exposure in patients subjected to renal transplantation.

It is well known that tacrolimus is primarily metabolised in the intestine and liver by the CYP3A family, especially its CYP3A4 and CYP3A5 members, and is a substrate of P-glycoprotein efflux pump [1, 3]. CYP3A is responsible for > 90% of tacrolimus metabolic elimination [18]. CYP3A4 accounts for 30% of the total cytochrome P450 activity in liver and 70% in small intestines [19]. It was reported that CYP3A5 was expressed at higher levels than CYP3A4 in extra hepatic tissue, such as in the small intestine, colon, lung, oesophagus, kidney, adrenal gland, anterior pituitary, breast, prostate and polymorphonuclear leukocytes [20]. The majority of compounds (tacrolimus, cyclosporine) that are substrates for CYP3A4, are also metabolized by CYP3A5, usually with a higher catalytic efficiency. Therefore, CYP3A5 is the predominant enzyme for metabolism of tacrolimus, with CYP3A4 contributing [21].

The efflux transporter P-glycoprotein also plays a major role in the pharmacokinetics of tacrolimus [21]. P-glycoprotein was found in enterocytes where it decreases intracellular concentrations of tacrolimus, by pumping them back into the lumen of the small intestine. P-glycoprotein also transports calcineurine inhibitors across membranes of hepatocytes and kidney cells, as well as lymphocytes. About 75% of interpatient variability in cyclosporine clearance could be explained by variation of both CYP3A4 activity in the liver, and expression of P-glycoprotein in enterocytes [22].

Polymorphisms of genes which encode previously mentioned most important enzymes and transporter for tacrolimus metabolism and elimination can have significant influence on oral bioavailability of this drug and its blood concentrations [10]. Therefore, larger doses of the drug (2–16 mg) were needed for the patients in order to get significantly higher tacrolimus trough concentrations. On the other hand, our results showed that patients who were treated with less than 2 mg of tacrolimus/day (0.024 ± 0.006 mg/kg/day) had tacrolimus C/D ratio larger than 150 ng/mL/mg/kg, and vice versa. When taking into account target tacrolimus concentrations, there are authors who consider that the higher C/D ratio obtained, the slower metabolic efficiency can be expected and, consequently, lower tacrolimus dose is required [5]. Therefore, in all patients who had tacrolimus C/D ratio over 150 ng/mL/mg/kg, the examination of genetic polymorphism was performed in order to show its influence on tacrolimus metabolism.

Most of these patients (84.62%) had CYP3A5 *3*3 allele (gene mutant, non-expressers for enzyme CYP3A5). It is interesting to mention that Đorđević et al. [23] showed that in Serbian population, in 140 healthy volunteers, 84.7% had *3*3 gene variant. It had already been shown that, after the equal dose of this drug, the patients with CYP3A5 *3 allele often have higher blood concentrations of tacrolimus in comparison to the patients who have CYP3A5 *1 allele (CYP3A5 *1*1/*1*3) [9]. Considering that CYP3A5 enzyme is dominant in tacrolimus metabolism, it can be expected that many patients in our population tend to have higher tacrolimus blood concentrations after empirical treatment with its usual dosage in the early period after kidney transplantation. Most of the studies confirmed that carriers of CYP3A5 *3*3 genotype require lower doses of tacrolimus [24, 25]. When the given doses were equal, in order to maintain drug levels in optimal range, it turned out that the carriers of CYP3A5 *1*1/*1*3 genotype had 1.5–2-fold higher TTC in comparison to CYP3A5 *3*3 genotype [24, 25].

All the patients in our study had CYP3A4 *1*1/*1*1B (gene non-mutant, expressers for enzyme CYP3A4), which is associated with the functional state of enzyme CYP3A4. The other authors showed that enzyme CYP3A4 is predominantly active in Caucasians in comparison to Asians, Mexicans and African-Americans. The presence of this polymorphism in Caucasians ranged from 90 to 98% [26, 27]. Some recent studies demonstrated that CYP3A4 polymorphism, resulting from the A > G substitution at position 392, referred to as CYP3A4*1B (CYP3A4 – 392 GG) allele, consequently caused a diminished enzymatic activity and, thus, reduced tacrolimus clearance [26].

Regarding ABCB1 C3435T gene, 30.77% of our patients had a mutant gene (TT variant, which is associated with the diminished activity of P-glycoprotein). P-glycoprotein, which is encoded by the ABCB1 gene, is a large ATP-dependent transmembrane protein involved in the extracellular efflux of tac-

rolimus [10]. The efflux pump is responsible for the efflux of the already absorbed tacrolimus from enterocytes back into the intestinal lumen and, therefore, reduces its bioavailability. The genetic polymorphism of P-glycoprotein is associated with the reduced function of this efflux pump and, consequently, the increased tacrolimus absorption and blood concentration. The most extensively investigated SNPs of ABCB1 are 3435C > T (rs1045642) in exon 26, 1236C > T (rs128503) in exon 12, and 2677G > T/A (rs2032582) in exon 21 [28]. It was shown that the patients who had wild-type genotype ABCB1 3435C > T (CC) had stable tacrolimus blood concentration, while the patients with TT variants ABCB1 had up to 60% higher tacrolimus blood levels [29], because TT genotype a expressed lower intestinal activity of P-glycoprotein. Consequently it could be supposed that better absorption of tacrolimus and lower daily dose would be required in these patients [18]. Gene variants for ABCB1 C3435T CC and CT are associated with normal activity of P-glycoprotein [30].

According to our study, among 211 patients who were subjected to renal transplantation, about 25% had both non-functional CYP3A5 *3*3 and non-functional ABCB1 C3435T (TT) allele [31]. On the other hand, in our previous retrospective case series study, 26.6 % of renal transplant recipients had tacrolimus blood concentration values equal to and lower than 5 ng/mL [13], similar to the results obtained in this study. Since the number of the patients with high tacrolimus C/D ratio was small, definite conclusions cannot be made. Some factors, other than genetic polymorphism, also led to significantly higher tacrolimus C/D ratio. Therefore, we may assume slower elimination efficiency and, consequently, the requirement for lower tacrolimus dose in these patients. It is in accordance with a widely accepted attitude that numerous factors are contributors to the high tacrolimus pharmacokinetic variability in this category of patients.

The limitation of the study relates to the small sample size of renal transplant recipients who had genotyping of CYP3A5 and CYP3A4 enzymes performed, as well as the transporter P-glycoprotein. Also, this study does not taken into account other variables that can affect the TTC and tacrolimus C/D ratio.

Conclusion

The correlation between tacrolimus daily dose per body weight and tacrolimus C/D ratio was very strong in renal transplant recipients in our study. Tacrolimus C/D ratio greater than 150 ng/mL/mg/kg is the cut-off value in patients subjected to renal transplantation which might indicate the patients who are poor CYP3A5 metabolizers and/or with dysfunctional P-glycoprotein. Therefore, genotyping of these genes in renal transplant recipients is beneficial in order to emphasize the necessity of the reduction of the initial tacrolimus dose which would, consequently, decrease the risk of achieving tacrolimus concentrations over the therapeutic range immediately after transplantation. Since numerous factors also contribute to the variability of tacrolimus blood concentrations, a greater number of studies examining the relationship between the polymorphism of genes and clinical endpoints will be needed. As a result, cost/benefit analysis could be done and, therefore, genetic examination, prior transplantation, justified.

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