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Clinical utility of ABCB1 single nucleotide polymorphism on tacrolimus dose requirements in Egyptian liver transplant patients

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Abstract

Background: Liver transplantation (LT) is the only effective radical cure for all types of end-stage liver diseases. Major advances have been made in the field of liver transplantation due to improvements in surgical techniques and organ conservation as well as optimization of intensive care and immunosuppressive management. We aimed to assess the influence of ABCB1 gene polymorphism of liver transplant recipients on blood level and dose requirements of oral tacrolimus, in an attempt to help in designing an individualized tacrolimus regimen for Egyptian liver transplant recipient. The study included 25 liver transplant recipients and their respective 25 donors. All subjects of this study were subjected to full medical history, clinical evaluation, laboratory investigations, and ABCB1 gene polymorphism evaluation by RT-PCR. Tacrolimus concentration was evaluated for all the recipients during the first 3 months post transplantation.

Results: The present study revealed that the presence of CC genotype was significantly correlated to the effect on tacrolimus C/D ratio and weight-adjusted tacrolimus dose during the first week of the first and 2nd months ($Z = -2.108$, $P < 0.05$) but not the 3rd month post transplantation (p-value >0.05). Subjects carrying CC genotype required higher doses of tacrolimus to achieve the desired trough levels compared to subjects carrying CT and TT genotypes. The same effect was observed over the whole period of the study but the results were statistically non-significant (p-value>0.05). Recipients who received liver tissue from donors carrying CC genotype also required higher doses of tacrolimus and reached lower levels of blood tacrolimus trough levels.

Conclusion: The present study revealed that ABCB1 CC genotype of both recipients and donors of liver transplantation was significantly associated with increased required tacrolimus dose early after liver transplantation reaching statistically significant level in the first week of the first and second months.

Keywords: ABCB1 single nucleotide, Polymorphism tacrolimus dose, Liver transplant

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Background

Liver transplantation (LT) is the only effective radical cure for all types of end stage liver diseases providing new hope for these patients. Immunosuppressant is the main preventive and treatment measure for organ transplant rejections. The appropriate use of immunosuppressant is directly related to the survival of the liver transplant recipients [1].

Tacrolimus is an immunosuppressant widely used in liver transplant patients. Tacrolimus is a calcineurin inhibitor, which is an enzyme that activates T-cells of the immune system. Due to its narrow therapeutic index and high inter- and intra-individual pharmacokinetic variability, the administration regimens of this drug need to be closely monitored [2]. The optimal trough levels were expected to be between 5 and 10 ng/ml for a better survival rate after liver transplantation [3].

Tacrolimus is a metabolic substrate for (CYP450) 3A enzymes in particular CYP3A5. The efflux of tacrolimus is through P-glycoprotein (P-gp) transporter, which together with CYP3A determines tacrolimus oral clearance [4].

Physiologically, P-gp is present on the surface of biliary canalicular hepatocytes, luminal surface of columnar epithelial cells of the lower gastrointestinal tract (GIT), liver, pancreas, small and large intestines, jejunum, and colon. P-gp alters the pharmacokinetics of some drugs including tacrolimus by reducing their intestinal absorption while enhancing their biliary excretion through the liver and tubular excretion in the kidney [5].

P-gp is encoded by the ABCB1 gene. Numerous single nucleotide polymorphisms occur in coding and non-coding regions which might influence mRNA expression and protein translation and folding, and finally affect drug pharmacokinetic characteristics [6].

Many studies revealed that substitution of cytosine (C) by thymine (T) in the ABCB1 gene in exon 27 was connected with the change of expression and activity of P-gp [7].

Aim of the work

The aim of the present work was to assess the influence of ABCB1 gene polymorphism of liver transplant recipients and their respective donors on blood level and dose requirements of oral tacrolimus, in an attempt to help in designing an individualized tacrolimus regimen for Egyptian liver transplant recipient.

Methods

This study is a cross-sectional study which was conducted from February 2020 to October 2020 on twenty-five (25) liver transplant recipients and their respective donors. They were recruited from Ain Shams Center of Organ Transplantation (ASCOT). An informed verbal consent was taken from all participants. The study protocol was approved by the Research Ethics Committee at Ain Shams University Faculty of Medicine.

Liver transplantation recipients (*n*=25)

This group included twenty-five (25) patients who had liver transplantation. They were 20 males and 5 females with age ranged from 36 to 67 years.

Liver transplantation donors (*n*=25)

This group included respective donors of liver transplant recipients; they were 18 males and 7 females with age ranged from 18 to 41 years. Subjects who had acute rejection or graft failure, less than 18 years old, and those who developed tacrolimus-related complications early in the post transplantation period necessitating change of immunosuppressant regimen were excluded from our study.

All individuals in this study were subjected to the following

Full medical history, clinical evaluation, and laboratory investigations in the form of total and direct bilirubin, urea, and creatinine. ALT, AST, total protein and albumin, CBC, INR, determination of ABCB1 gene polymorphism using real time-polymerase chain reaction technique (RT-PCR), tacrolimus concentration/dose ratio (C/D ratio) calculated with trough concentration, and weight-standardized 24-h tacrolimus dose (mg/kg/d).

Sampling

Seven milliliters of venous blood were collected 2 h before the next tacrolimus dose under complete aseptic precautions divided into three types of tubes as follows:

Citrate vacutainer for assay of coagulation profile, plain tube containing gel for serum separation to perform AST, ALT, total protein, total and direct bilirubin, tri-potassium ethylene diamine tetra acetate "k3 EDTA" vacutainer for complete blood count (CBC), TAC concentration, and RT-PCR.

Blood collected in plain tubes was left for 20 min to clot then centrifuged at 2000–3000 RPM for 10 min. The sera were separated for assay of liver profile and serum creatinine. EDTA vacutainer collected for assay of ABCB1 polymorphism were stored at −70 °C as whole blood till the time of analysis. Repeated freezing/thawing of samples was avoided.

Methods

a. Analytical methods

1. Assay of tacrolimus trough level

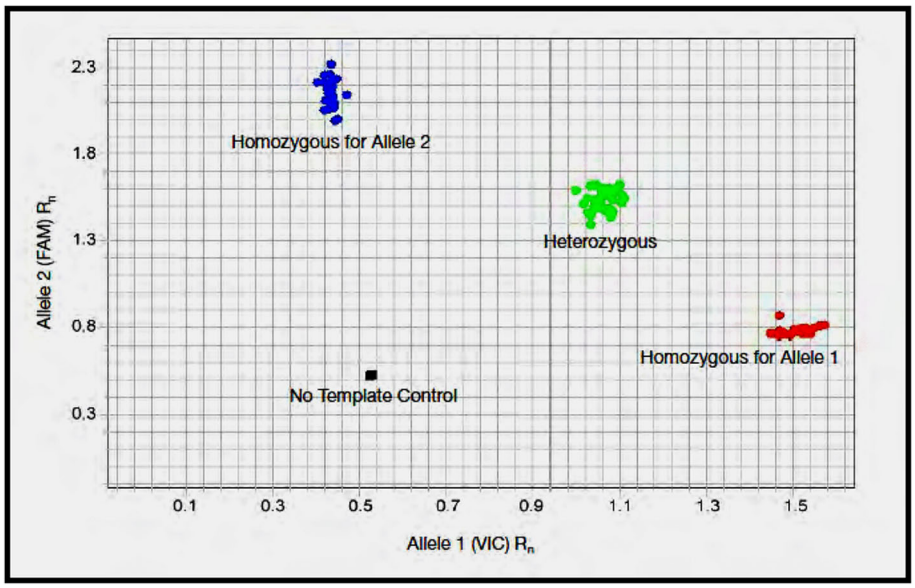

Fig. 1 Allelic discrimination plot

Done by Abbott ARCHITECT tacrolimus competitive immunoassay method with chemiluminescent microparticle immunoassay (CMIA) technology. According to the manufacturer's instructions, blood specimens were pretreated by rapidly vortex mixing of 200 μL of EDTA whole blood with 200 μL of precipitation reagent, followed by centrifugation to obtain clear supernatant for analysis. Tacrolimus in the specimen competes with tacrolimus acridinium-labeled conjugate for the available binding sites on the anti-tacrolimus antibody-coated paramagnetic microparticles. The resulting chemiluminescent signal is indirectly related to the amount of tacrolimus in the specimen.

2. Assay of ABCB1polymorphism by real-time PCR

Detection of ABCB1 polymorphism (rs1045642) was performed by TaqMan real-time PCR kit supplied by Thermo Scientific[1]. The technique was done in three main steps: extraction of genomic DNA from peripheral blood leucocytes in an EDTA whole blood sample, amplification of the extracted DNA, and allelic discrimination by real-time PCR.

Principle

TaqMan genotyping assays genotype SNPs using the 5′ nuclease assay for amplifying and detecting specific SNP alleles in purified genomic DNA samples. Each TaqMan genotyping assay contains two primers for amplifying the sequence of interest and two TaqMan probes for detecting alleles. The presence of two probe pairs in each reaction allows genotyping of the two possible variant alleles at the SNP site in a DNA target sequence. The genotyping assay determines the presence or absence of a SNP based on the change in fluorescence of the dyes associated with the probes.

Result interpretation

A substantial increase in FAM dye florescence only indicates homozygosity for allele 1 (Wild allele), substantial increase in VIC dye florescence only indicates homozygosity for allele 2 (Mutant allele), and substantial increase in both VIC and FAM dye fluorescence indicates allele 1 allele 2 heterozygosity (Fig. 1).

b. Statistical methods

Data were collected, revised, coded, and entered to the Statistical Package for Social Science (IBM SPSS) version 23 for data analysis.

The Friedman test used is a non-parametric statistical test used to detect differences in treatments across multiple test attempts. The chi-square test (χ^2) is applied to study the association between each 2 variables (Pearson chi-square) or comparison between 2 independent groups as regards the categorical data. Mann–Whitney U test is a test to compare nonparametric data.

Results

The results obtained in the present study are shown in Tables 1, 2, 3, 4, 5, 6, 7, and 8.

[1]** Thermo Scientific: 168 Third Avenue, Waltham, MA, USA 02451.

Table 1 Comparison between recipient and donors regarding ABCB1 genotype and its allele distribution (by chi-square test)

		Recipient		Donor		χ^2	P-value	Sig.
		No.	%	No.	%			
ABCB1	**CC**	11	44.0%	15	60.0%	1.377	0.502	NS
	CT	12	48.0%	9	36.0%			
	TT	2	8.0%	1	4.0%			
Allele	**C**	34	68.0%	39	78.0%	1.268	0.260	NS
	T	16	32.0%	11	22.0%			

Table 1 demonstrates descriptive and comparative statistics of the genotype and allele frequencies of ABCB1 gene polymorphism (rs1045642) between donors and recipients. The CC genotype (wild type) constituted 60% and 44% for the donors and recipients respectively, while TT genotype (Mutant type) constituted 4% of the donors and 8% of the recipients. Thirty-six (36%) of donors and 48% of recipients had CT genotype.

Follow-up for concentration/dose ratio (C/D), tacrolimus dose, and tacrolimus concentration among the recipients during the first 4 weeks post transplantation are shown in Table 2. The results of follow-up for C/D ratio and required tacrolimus dose in relation to genotype of the recipient was significant in the first week of the first month post transplantation (p-value <0.05). During the 1st week, C/D ratio median value was significantly lower in subjects with CC genotype (median (IQR) = 48) compared to subjects with TT and CT genotypes (median (IQR) = 134.9). The dose requirements needed to reach the trough level was significantly higher in subjects with CC genotype compared to subjects with TT and CT genotypes ranging (0.01–0.7mg/kg/day) and (0.01–0.09mg/kg/day) respectively (p-value <0.05). Follow-up of C/D ratio, dose, and concentration of tacrolimus in the next 3 weeks was statistically non-significant (p-value>0.05) (Table 3).

Recipients group were followed up in time intervals of 2 months and 3 months post transplantation; C/D ratio was significantly lower and required dose was significantly higher in recipients carrying CC genotype median = 63.5 compared to C/D ratio of median = 126.5 for the recipients carrying CT or TT genotype during the 2nd month post transplantation, while the effect of genotype was statistically non-significant during the 3rd month post transplantation (Table 4).

The donors' group's genotype effect on tacrolimus dose, concentration, and C/D ratio during the 1st, 2nd, and 3rd months is illustrated in Tables 5 and 6. C/D ratio during the 1st week post-transplantation was significantly higher in subjects who received liver tissue from TT and CT genotype compared to subjects who received liver tissue CC genotype (p-value <0.05), whereas the results were non-significant during the following 3 months.

Study subjects were further subdivided into 4 groups according to genotype of donor and recipient. Groups 1 and 2 are compared as regards C/D ratio, tacrolimus dose, and trough level during the first 3 months post transplantation as shown in Table 7. In these groups, the recipient genotype is CC while donor genotype is either CC or CT/TT for groups 1 and 2 respectively. C/D ratio of group 1 (median (IQR) = 44.1) compared to C/D ratio of group 2 (median (IQR) = 113) was statistically significant during the 1st week post transplantation. There was no statistical significance during the next 3 months between the 2 groups.

In groups 3 and 4, the recipient genotype is CT/TT while donor genotype is either CC or CT/TT respectively. C/D ratio, tacrolimus dose, and trough level during the 1st 3 months post transplantation in Table 8 show no statistical significance between the 2 groups.

P-value >0.05, non-significant (NS); P-value <0.05, significant (S); P-value< 0.01, highly significant (HS)

P-value >0.05, non-significant (NS); P-value <0.05, significant (S); P-value< 0.01, highly significant (HS)

P-value >0.05, non-significant (NS); P-value <0.05, significant (S); P-value< 0.01, highly significant (HS)

Discussion

Tacrolimus (also known as FK506), a calcineurin inhibitor (CNI), is the cornerstone in the immunosuppressive

Table 2 Follow-up for C/D, tacrolimus dose, and tacrolimus concentration among recipients during the first 4 weeks post transplantation (Friedman test)

		1st week No. = 25	2nd week No. = 25	3rd week No. = 25	4th week No. = 25	Test value	*P*-value	Sig.
C/D	**Median (IQR)**	98.8 (46–140)	95.2 (66.3–122.4)	105 (66–131.4)	117.3 (91.2–134.7)	1.289	0.732	NS
	Range	22.5–350	33.75–740	38.16–310	62.1–326.0			
Dose (mg/kg/day)	**Median (IQR)**	0.05 (0.03–0.07)	0.06 (0.04–0.08)	0.06 (0.04–0.08)	0.05 (0.04–0.07)	4.915	0.178	NS
	Range	0.01–0.7	0.01–4	0.02–5.2	0.02–4.5			
Trough level (ng/dl)	**Median (IQR)**	4.25 (2.40–5.30)	5.1 (3.9–6.8)	5.7 (4.5–6.9)	5.7 (5–6.5)	5.331	0.004	HS
	Range	0.9–7.9	2.7–9.6	2.9–15	1.4–14.5			

Table 3 Relation of ABCB1 genotype and C/D ratio and tacrolimus dose and concentration among the recipient group during the first month post transplantation (Mann–Whitney test)

		ABCB1		Z	*P*-value	Sig.
		CC	CT+TT			
C/D 1st wk	**Median (IQR)**	48 (39–100)	134.95 (76.6–158)	−2.108	0.035	S
	Range	32–221	22.5–350			
Tac dose (mg/kg/day)	**Median (IQR)**	0.07 (0.05–0.1)	0.04 (0.03–0.06)	−2.095	0.036	S
	Range	0.01–0.7	0.01–0.09			
Trough level (ng/dl)	**Median (IQR)**	4.14 (1.8–4.9)	4.43 (2.6–5.8)	−0.904	0.366	NS
	Range	1–6.8	0.9–7.9			
C/D 2nd wk	**Median (IQR)**	75.5 (66–113.75)	100.25 (75–147.2)	−1.123	0.262	NS
	Range	54.5–170	33.75–740			
Tac dose (mg/kg/day)	**Median (IQR)**	0.06 (0.05–0.08)	0.06 (0.04–0.08)	−0.359	0.719	NS
	Range	0.03–4	0.01–1.5			
Trough level (ng/dl)	**Median (IQR)**	4.45 (3.7–6.8)	5.6 (3.9–7.4)	−0.411	0.681	NS
	Range	2.8–8.5	2.7–9.6			
C/D 3rd wk	**Median (IQR)**	105 (60–116)	106.3 (66.24–137.5)	−0.876	0.381	NS
	Range	38.16–169	57.3–310			
Tac dose (mg/kg/day)	**Median (IQR)**	0.05 (0.04–0.09)	0.06 (0.03–0.07)	−0.358	0.720	NS
	Range	0.02–4.5	0.02–0.09			
Trough level (ng/dl)	**Median (IQR)**	5.2 (3.6–6.5)	5.8 (4.8–7.8)	−0.849	0.396	NS
	Range	3.16–10.4	2.9–15			
C/D 4th wk	**Median (IQR)**	91.2 (70.0–123.5)	122.25 (110–162)	2.1325	0.063	NS
	Range	62.1–167	72.32–326.0			
Tac dose (mg/kg/day)	**Median (IQR)**	0.06 (0.04–0.09)	0.06 (0.04–0.07)	−0.111	0.912	NS
	Range	0.02–5.2	0.03–0.1			
Trough level (ng/dl)	**Median (IQR)**	5.7 (3–6.1)	5.85 (5–8.4)	−1.233	0.217	NS
	Range	1.4–7.75	2.2–14.5			

Table 4 Relation of ABCB1 genotype and C/D ratio, tacrolimus dose, and trough levels among the recipient group 2 months and 3 months post transplantation (Mann–Whitney test)

ABCB1		CC	CT+TT	Z	*P*-value	Sig.
2nd month						
Dose	**Median (IQR)**	0.1 (0.07–0.1)	0.05(0.03–0.08)	−1.902	0.050	S
	Range	0.04–0.1	0.02–0.1			
Trough level	**Median (IQR)**	5.3(3.8–10.6)	6.65(6.05–7.8)	−0.797	0.426	NS
	Range	2.9–12.9	2.2–14.6			
C/D	**Median (IQR)**	63.5(49–106)	126.5(88.25–207.5)	−2.154	0.031	S
	Range	47–143	55–486			
3rd month						
Dose	**Median (IQR)**	0.08(0.05–0.09)	0.05(0.03–0.07)	−1.508	0.132	NS
	Range	0.02–0.1	0.01–0.1			
Trough level	**Median (IQR)**	4.95(4.5–7.4)	5.8(3.3–7)	−0.188	0.851	NS
	Range	3.2–8.8	1.4–9.1			
C/D	**Median (IQR)**	76.5(61–176)	122(84.5–147.5)	−0.469	0.639	NS
	Range	32–225	47–205			

P-value >0.05, non-significant (NS); *P*-value <0.05, significant (S); *P*-value< 0.01, highly significant (HS)

Table 5 Relation of ABCB1 genotype and C/D ratio and tacrolimus dose and concentration among the donors' group in relation to ABCB1 genotype (Mann–Whitney test)

		ABCB1		U test	P-value	Sig.
		CC	CT+TT			
C/D 1st wk	**Median (IQR)**	70 (34.5–132.5)	131.7 (100–160.3)	−2.164	0.030	S
	Range	22.5–350	48–294.4			
Tac dose (mg/kg/day)	**Median (IQR)**	0.06 (0.04–0.07)	0.04 (0.03–0.09)	−0.950	0.342	NS
	Range	0.01–0.1	0.01–0.7			
Trough level (ng/dl)	**Median (IQR)**	4.14 (2.10–4.60)	5.10 (2.60–6.70)	−1.526	0.127	NS
	Range	0.9–5.8	1–7.9			
C/D 2nd wk	**Median (IQR)**	95.2 (66.3–122.4)	93.85 (56–147.2)	−0.055	0.956	NS
	Range	33.75–187.5	54.5–740			
Tac dose (mg/kg/day)	**Median (IQR)**	0.06 (0.04–0.08)	0.07 (0.05–0.1)	−1.092	0.275	NS
	Range	0.03–0.1	0.01–4			
Trough level (ng/dl)	**Median (IQR)**	4.60 (3.70–8.00)	5.10 (4.45–6.20)	−0.361	0.718	NS
	Range	2.7–9.6	2.7–7.4			
C/D 3rd wk	**Median (IQR)**	78 (61.6–116)	125.75 (98.6–169)	−1.609	0.108	NS
	Range	38.16–310	57.3–200.3			
Tac dose (mg/kg/day)	**Median (IQR)**	0.06 (0.04–0.08)	0.06 (0.04–0.08)	−0.168	0.867	NS
	Range	0.02–0.1	0.02–5.2			
Trough level (ng/dl)	**Median (IQR)**	5.20 (3.60–6.50)	5.75 (5.20–7.80)	−0.971	0.331	NS
	Range	2.9–9.3	3.16–15			
C/D4th wk	**Median (IQR)**	122 (99.5–162)	99.45 (79.0–123.5)	0.998	0.318	NS
	Range	62.1–250	70.0–326.0			
Tac dose (mg/kg/day)	**Median (IQR)**	0.04 (0.03–0.06)	0.06 (0.04–0.07)	−0.926	0.355	NS
	Range	0.02–0.2	0.02–4.5			
Trough level (ng/dl)	**Median (IQR)**	5.60 (5.00–6.50)	6.10 (4.17–8.50)	−0.694	0.487	NS
	Range	2.2–8.4	1.4–14.5			

P-value >0.05, non-significant (NS); *P*-value <0.05, significant (S); *P*-value< 0.01, highly significant (HS)

Table 6 Relation of ABCB1 genotype and C/D ratio, tacrolimus dose, and trough levels among the donor group 2 months and 3 months post transplantation (Mann–Whitney test)

ABCB1		CC	CT+TT	U test	*P*-value	Sig.
2nd month						
Dose	**Median (IQR)**	0.05(0.04–0.08)	0.08(0.04–0.1)	−0.812	0.417	NS
	Range	0.02–0.1	0.03–0.1			
Trough level	**Median (IQR)**	6.65(5.9–8.1)	6.05(5.1–9.85)	−0.222	0.824	NS
	Range	2.2–10.6	3.8–14.6			
C/D	**Median (IQR)**	108(82.5–167)	86(54.5–184.5)	−0.355	0.722	NS
	Range	49–213	47–486			
3rd month						
Dose	**Median (IQR)**	0.05(0.03–0.06)	0.08(0.05–0.1)	−1.878	0.060	NS
	Range	0.01–0.07	0.02–0.1			
Trough level	**Median (IQR)**	5.4(2.1–8.2)	5.35(4.5–6.95)	−0.089	0.929	NS
	Range	1.4–9.1	3.2–7.4			
C/D	**Median (IQR)**	142.5(71–150)	90.5(59.5–119.75)	−1.422	0.155	NS
	Range	47–225	32–205			

P-value >0.05, non-significant (NS); *P*-value <0.05, significant (S); *P*-value< 0.01, highly significant (HS)

Table 7 C/D ratio in correlation with different genotype of donor when recipient genotype is CC (Mann–Whitney test)

		Group 1 (CC/CC) No. = 7	Group 2 (CC/CT or TT) No. = 4	U test	*P*-value	Sig.
1st wk						
C/D ratio	**Median (IQR)**	44.1(34.5–70)	113(74–173.5)	−2.268	0.023	S
	Range	32–75.2	48–221			
Dose	**Median (IQR)**	0.07(0.05–0.09)	0.07(0.02–0.4)	0.000	1.000	NS
	Range	0.05–0.1	0.01–0.7			
Trough level	**Median (IQR)**	4.14(2.4–4.4)	3.55(1.4–6.05)	−0.189	0.850	NS
	Range	1.6–4.9	1–6.8			
2nd wk						
C/D	**Median (IQR)**	92.5(66.3–122.4)	65.45(55.25–94.33)	−1.323	0.186	NS
	Range	66–170	54.5–113.75			
Dose	**Median (IQR)**	0.06(0.04–0.08)	0.08(0.06–2.05)	−1.245	0.213	NS
	Range	0.03–0.08	0.05–4			
Trough level	**Median (IQR)**	4.2(3.7–8.4)	4.78(3.63–5.3)	−0.379	0.705	NS
	Range	3.7–8.5	2.8–5.5			
3rd wk						
C/D	**Median (IQR)**	78(53.6–112)	125.3(82.5–157.3)	−1.136	0.256	NS
	Range	38.16–116	60–169			
Dose	**Median (IQR)**	0.06(0.04–0.09)	0.07(0.04–2.64)	−0.190	0.849	NS
	Range	0.02–0.1	0.02–5.2			
Trough level	**Median (IQR)**	5.2(3.6–6.5)	5.2(3.88–8.1)	0.000	1.000	NS
	Range	3.3–9	3.16–10.4			
4th wk						
C/D	**Median (IQR)**	99.5(63–141)	107.35(85.1–285.75)	−0.756	0.450	NS
	Range	62.1–167	79–448			
Dose	**Median (IQR)**	0.05(0.04–0.09)	0.04(0.02–2.28)	−0.579	0.563	NS
	Range	0.04–0.2	0.02–4.5			
Trough level	**Median (IQR)**	5.85(5.2–6.5)	3.51(2.13–5.14)	−1.492	0.136	NS
	Range	3–7.75	1.4–6.1			
2 months						
Dose	**Median (IQR)**	0.1(0.04–0.1)	0.09(0.07–0.1)	−0.232	0.817	NS
	Range	0.04–0.1	0.07–0.1			
Trough level	**Median (IQR)**	5.9(2.9–10.6)	4.7(3.8–12.9)	−0.218	0.827	NS
	Range	2.9–10.6	3.8–12.9			
C/D	**Median (IQR)**	73(49–106)	54(47–143)	−0.218	0.827	NS
	Range	49–106	47–143			
3 months						
Dose	**Median (IQR)**	0.05(0.02–0.07)	0.09(0.08–0.1)	−1.964	0.050	NS
	Range	0.02–0.07	0.08–0.1			
Trough level	**Median (IQR)**	5(4.5–8.8)	4.9(3.2–7.4)	−0.655	0.513	NS
	Range	4.5–8.8	3.2–7.4			
C/D	**Median (IQR)**	176(71–225)	61(32–82)	−1.528	0.127	NS
	Range	71–225	32–82			

$P > 0.05$, non-significant; $P < 0.05$, significant; $P < 0.01$, highly significant

Table 8 C/D ratio in correlation with different genotype of donor when recipient genotype is CT or TT (Mann–Whitney test)

		Group 3 (CT or TT/CC) No. = 8	Group 4 (CT or TT/CT or TT) No. = 6	U test	*P*-value	Sig.
1st wk						
C/D ratio	**Median (IQR)**	115.65(55.55–150.75)	138.7(113.5–160.3)	−0.775	0.439	NS
	Range	22.5–350	72–294.4			
Dose	**Median (IQR)**	0.05(0.04–0.06)	0.04(0.03–0.05)	−0.589	0.556	NS
	Range	0.01–0.06	0.02–0.09			
Trough level	**Median (IQR)**	4.18(1.95–4.95)	5.6(4.2–6.7)	−1.678	0.093	NS
	Range	0.9–5.8	2.6–7.9			
2nd wk						
C/D	**Median (IQR)**	100.25(65.1–126)	121.2(92.5–176.4)	−0.711	0.477	NS
	Range	33.75–187.5	54.7–740			
Dose	**Median (IQR)**	0.06(0.04–0.08)	0.07(0.04–0.1)	−0.393	0.694	NS
	Range	0.04–0.1	0.01–1.5			
Trough level	**Median (IQR)**	5.35(3.5–7.75)	5.65(5–6.3)	−0.065	0.948	NS
	Range	2.7–9.6	2.7–7.4			
3rd wk						
C/D	**Median (IQR)**	76.35(63.92–124)	125.75(98.6–196)	−0.904	0.366	NS
	Range	58–310	57.3–200.3			
Dose	**Median (IQR)**	0.07(0.05–0.07)	0.05(0.04–0.08)	−0.327	0.744	NS
	Range	0.03–0.1	0.03–0.1			
Trough level	**Median (IQR)**	5.35(4–7.7)	6.3(5.5–7.8)	−1.033	0.302	NS
	Range	2.9–9.3	5.2–15			
4th wk						
C/D	**Median (IQR)**	128.6(119.65–191)	114.45(91.4–133)	−1.291	0.197	NS
	Range	110–250	72.32–326.4			
Dose	**Median (IQR)**	0.04(0.02–0.06)	0.07(0.05–0.07)	−1.899	0.058	NS
	Range	0.02–0.07	0.04–0.09			
Trough level	**Median (IQR)**	5.15(4.75–6.55)	7.5(6.1–8.5)	−2.200	0.028	S
	Range	2.2–8.4	5.46–14.5			
2 months						
Dose	**Median (IQR)**	0.04(0.03–0.08)	0.05(0.03–0.1)	−0.575	0.565	NS
	Range	0.02–0.08	0.03–0.1			
Trough level	**Median (IQR)**	6.7(6.4–8.1)	6.2(5.9–6.8)	−0.568	0.570	NS
	Range	2.2–8.4	5.5–14.6			
C/D	**Median (IQR)**	140(94–202)	113(59–226)	−0.081	0.935	NS
	Range	82.5–213	55–486			
3 months						
Dose	**Median (IQR)**	0.04(0.03–0.06)	0.05(0.04–0.07)	−0.818	0.414	NS
	Range	0.01–0.07	0.02–0.1			
Trough level	**Median (IQR)**	5.8(1.5–8.2)	5.8(4.5–6.9)	−0.082	0.935	NS
	Range	1.4–9.1	4.5–7			
C/D	**Median (IQR)**	140(70–150)	112.5(99–127)	−0.407	0.684	NS
	Range	47–150	58–205			

$P > 0.05$, non-significant; $P < 0.05$, significant; $P < 0.01$, highly significant
Group 1 (CC/CC), group 2 (CC/CT or TT), group 3 (CT or TT/CC), and group 4 (CT or TT/CT or TT)

regimen post liver transplantation. By suppressing calcineurin activity, interleukin-2 (IL-2) production by T-cells is also reduced which affects proliferation and maturation of T-cells exerting immunosuppression effect. Therapeutic use of tacrolimus is complicated by its narrow therapeutic index, interpatient pharmacokinetics' variability, and the risk of drug interactions with co-administrated medications [8].

Passage of the drug across biological membranes is mostly regulated by membrane transporters. These membrane proteins determine the distribution of different drugs throughout the body, and they are hence major determinants of drug pharmacokinetic/pharmacodynamics profile [9]. The oral bioavailability of tacrolimus varies greatly between individuals and largely depends on the activity of cytochrome P4503A (CYP3A) subfamily and P-glycoprotein (P-gp) which is an efflux transporter encoded by the MDR1/ABCB1 [adenosine triphosphate (ATP)-binding cassette subfamily B, member 1] gene.

The ABCB1 gene has been extensively studied for characteristic polymorphisms and it has been shown that many of these polymorphisms may be linked with the function of P-glycoprotein. About 50 SNPs for ABCB1 have been identified. The most frequently studied polymorphisms for ABCB1 gene are C1236T (rs1128503), G2677T/A (rs2032582), and C3435T (rs1045642) [10]. The possible influence of genetic polymorphisms of P-gp in transplant recipients have been indicated as one of the most important variables affecting the pharmacokinetics of immunosuppressive drugs [11].

The aim of the present work was to assess the influence of ABCB1 gene polymorphism (rs1045642) of liver transplant recipients and donors on blood level and dose requirements of oral tacrolimus, in an attempt to help in designing an individualized tacrolimus regimen for Egyptian liver transplant recipient. The study was conducted on 25 patients who received liver transplantation in liver transplantation unit at Ain Shams University Specialized hospitals and their 25 respective donors.

Liver transplant recipients in the present study were given the same drug formulation through the same route of administration; they all received oral tacrolimus in the form of immediate release Prograf ®.

Pediatric and elderly individuals over 65 years were excluded. This exclusion was to avoid variability in tacrolimus pharmacokinetics that might be introduced by age differences [12].

All individuals in this study were subjected to full medical history, clinical evaluation, and laboratory investigations in the form of total and direct bilirubin, urea, and creatinine. ALT, AST, total protein and albumin, CBC, INR, determination of ABCB1 gene polymorphism using real-time-polymerase chain reaction technique (RT-PCR), tacrolimus blood concentration using chemiluminescent microparticle immunoassay (CMIA) tacrolimus assay on the Abbott Architect® analyzer. Concentration/dose ratio (C/D ratio) calculated with trough concentration and weight-standardized 24-h tacrolimus dose (mg/kg/d).

In the present study, ABCB1 CC genotype was detected in 11 (44%) recipients and 14 (56%) were CT and TT genotype, while the donors with CC genotype were 15 (60%) and those with CT/TT genotype were 10 (40%), respectively.

The genotype distribution of our study was similar to a study by Abd El-Hakim et al. [13] who reported the presence of CT and TT genotypes in 26 recipients (54%) while CC genotype was detected in 22 subjects (45.8%) of the enrolled 48 subjects. On the other hand, Venuto et al. [14] reported that of 149 American patients, 57 (38.3%) were CC genotype while 92 patients (61.7%) were CT/TT genotype. Moreover, Denga et al. [15] who genotyped 136 Chinese liver transplant recipients for ABCB1 polymorphism also found that 52 subjects (38.2%) were CC genotype while 84 subjects (61.7%) were CT/TT genotypes.

Similarly, Bonate et al. [16] showed that genotype distribution differs between races (African, American and Caucasians) affecting required doses in order to attain comparable tacrolimus levels. Ethnic variations would explain the different genotype distribution reported by different studies.

In our study, tacrolimus daily dose was significantly increased to achieve adequate levels among recipients carrying ABCB1 CC genotype compared to recipients carrying CT and TT genotypes during the 1st week. Thus, C/D ratio was significantly lower in recipients carrying CC genotype compared to those carrying CT or TT genotypes.

Our study subjects were followed up for 3 months. Tacrolimus dose was significantly higher in recipients with CC genotype with concomitant decrease in C/D ratio during the first week of the 2nd month. These changes were also observed during 2nd, 3rd, and 4th weeks of the first month and also during the rest of the study period although not reaching statistical significance.

It is noteworthy that during the follow-up, seven recipients experienced tacrolimus side effects as hallucinations and tacrolimus resistance by the end of 1st month requiring change of the immunosuppressant. Out of these 7 recipients, 5 were CC genotype (71.4%) and the remaining were CT genotype. These patients required high doses of tacrolimus but failed to reach the sufficient trough level for the optimum immunosuppression.

These findings were similar to the results reported by Helal et al. [7] who also observed an increase in required

tacrolimus dose among recipients with CC genotype compared to CT and TT genotypes.

Staatz et al. [17] studied the effect of different genotype on tacrolimus pharmacokinetics in renal transplant patients and demonstrated a higher tacrolimus concentration and a lower dose requirement in patients with TT variant genotype than in those with the CC wild-type genotype denoting lower functional activity of P-glycoprotein in the variant genotype. This was also observed by Vafadari et al. [18] who reported that TT homozygous patients' T-cells have a less active efflux pump, hence more inhibition of the production of IL-2 resulting in a better graft survival and less graft rejection.

Moreover, in a study by Gérard et al. [19], 66 adult liver recipients receiving oral tacrolimus were included in this study. Data were collected from day 1 to day 25 post-transplantation showing an approximately 1.5-fold difference in tacrolimus estimated clearance between TT and CC recipients being higher in CC genotype.

On the other hand, Kurzawski et al. [20] found no significant difference reported for tacrolimus dose or C/D between the different ABCB1 (rs1045642) genotypes in a study held on 241 kidney transplant patients through the 1st year post transplantation.

These results were also reported by Suzuki et al. [21] who investigated a total of 80 consecutive living-donor liver transplant (LDLT) recipients on tacrolimus. Sixty (60) patients were completely followed for 7 days early after liver transplantation in order to evaluate the pharmacokinetics. No effect of ABCB1 polymorphism on the required dose was observed.

A similar non-significant result by [22] was also reported in meta-analysis carried out to evaluate how recipient ABCB1 (n = 318) genotypes influence tacrolimus pharmacokinetics till 1 month of transplantation. It was reported that the recipient ABCB1 3435 C > T polymorphism has no significant influence on tacrolimus pharmacokinetics till 1 month of transplant.

A follow-up study of 51 Caucasian patients by Provenzani et al. [23] at 1, 3, and 6 months after transplantation as regards the ABCB1 SNPs did not show any appreciable influence on tacrolimus dosing requirements.

In the previous mentioned studies, the difference in follow-up period could possibly be an explanation as prolonged follow-up is subjected to factors such as introduction of other immunosuppressant with subsequent reduction of tacrolimus while short period of follow-up could be affected by patients loss as a result of death from complications. Also, inter-individual variability is high as regards dose modifications to reach sufficient trough levels of about (8–10 ng/dl).

To evaluate the effect of the donor genotype on the recipient response, study subjects were further subdivided into 4 groups. Recipient is CC and donor is CC in group 1. Recipient genotype is CC and donor genotype is CT/TT in group 2. Recipient is CT/TT and donor is CC in group 3, recipient genotype is CT/TT, and donor genotype is CT/TT in group 4.

Our study shows statistical significance between group 1 and 2 during the first week post transplantation as regards C/D ratio. Decreased C/D ratio in group 1 intensifies the effect of combined effect of CC genotype of recipient and donor. Sixty (60%) of donors carried CC genotype. Their corresponding recipients required higher tacrolimus dose than those who received from CT or TT genotypes. Their drug concentration was also lower which was associated with a significantly lower levels of C/D compared to 40% who received liver transplantation from CT and TT genotypes. Otherwise, the genotype had no statistical significance during the follow-up at 2 and 3 months.

Both groups 3 and 4 show no statistical significance when compared as regards C/D ratio, tacrolimus dose, or trough levels during the 1st, 2nd, and 3rd months.

A study done by Gómez-Bravo et al. [24] evaluated the impact of ABCB1 genotypes of graft and patient on tacrolimus dosage requirement and on the incidence of acute rejection among adult Caucasian Spanish liver transplant recipients. In the 98 subjects of this study, no consistent evidence has been found that the ABCB1 genotype of either recipient or the graft has a significant influence on the distribution of tacrolimus or the incidence of acute rejection or other adverse events.

The clinical outcome at 2-year follow-up study by Glowacki et al. [25] on 209 French renal transplant recipients who received tacrolimus as the main immunosuppressant did not appear to be related to the donor or recipient ABCB1 3435C>T polymorphism and no difference was reported regarding the required dose between the subjects of the study.

The effect of recipient genotype was more prominent than that of the donor's because recipients with CC genotype showed an expression of P-gp not only in the liver but also in the duodenum endothelium, kidney, adrenal gland, and pancreas two times higher compared to individuals with TT and CT genotypes while the donor effect is only related to the transplanted liver; therefore, CC-genotyped recipients experience reduced intestinal absorption of orally administered drugs including tacrolimus and enhanced biliary excretion through the liver and tubular excretion in the kidney [26].

Conclusion

The present study revealed that ABCB1 CC genotype of both recipients and donors of liver transplantation was

significantly associated with increased required tacrolimus dose early after liver transplantation reaching statistically significant level in the first week of the first and second months. Further studies are recommended using a larger sample size and longer duration of follow-up.

Abbreviations

C/D ratio: Concentration dose ratio,; CYP450: Cytochrome P450; CMIA: Chemiluminescent microparticle immunoassay; CNI: Calcinurin inhibitor; IQR: Interquartile range; LT: Liver transplantation; P-gp: Permeability glycoprotein; RT-PCR: Real-time polymerase chain reaction

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Authors' contributions

AS drafted the manuscript; HA carried out the molecular genetic studies, participated in the sequence alignment, and participated in study design. MS carried out the molecular genetic studies, participated in the sequence alignment and performed statistical analysis. MM participated in the study design. The late EE made the study design. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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