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Original article

Effect of ABCB1 (3435C>T) and CYP3A5 (6986A>G) genes polymorphism on tacrolimus concentrations and dosage requirements in liver transplant patients

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ABSTRACT

Background: Tacrolimus (TAC) is an immunosuppressant used in organ transplant recipients. It is a substrate of drug transporter ABCB1 as well as of cytochrome P4503A (CYP3A).
Aim: To assess the influence of ABCB1 (3435 C>T) and CYP3A5 (6986 A>G) genes polymorphism of liver transplant donors and recipients on blood level and dose requirements of oral tacrolimus, to help in designing an individualized tacrolimus regimen for liver transplant recipients.
Subjects and methods: Forty-eight adult liver transplant recipients and their matching living donors were prospectively enrolled in this study. TAC doses and blood concentration were recorded on 1st, 2nd and 3rd days, after 1 and 2 weeks, and at 1, 3 and 6 months postoperatively using ultra performance liquid chromatography Tandem mass spectrometry. Genotyping of ABCB1 (3435C>T) and CYP450 3A5 (6986A>G) genes were determined by Polymerase chain reaction followed by restriction fragment length polymorphism and by TaqMan allelic discrimination assay techniques, respectively.
Results: Of the enrolled 48 recipients, CYP3A5*3/*3 and CYP3A5*1/*3 genotypes were detected in 18 (37.5%) and in 20 (41.7%) recipients respectively, while ABCB1 CT and TT genotypes were detected in16 (33.3%) and 10 (20.8%) recipients respectively. TAC daily dose was significantly increased among recipients carrying ABCB1 CC genotype compared to recipients carrying CT and TT genotypes during and after the first month postoperatively. During 1st, 2nd days and 2 weeks post-transplant, a significant increase of TAC concentration / dose ratio was observed among recipients carrying CYP3A5*3*3 genotype than recipients carrying 1*1* and 1*3* genotypes, and among recipients carrying ABCB1 CT and TT genotypes compared to those carrying CC genotype on 1st, 3rd days and at 3 months postoperatively.
Conclusions: ABCB1 and CYP3A5 genetic polymorphism is one of the factors influencing TAC pharmacokinetics, screening for these SNPs prior to liver transplantation might be helpful for individualization of tacrolimus treatment.

1. Introduction

Liver transplantation (LT) is the only effective radical cure for all types of end stage liver diseases and provides new hope for end stage liver disease 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 (TAC) is the backbone of immunosuppressive drugs used worldwide in organ transplantation, and characterized by a narrow therapeutic range and high inter-individual variability in its pharmacokinetics [2]. Over the years, TAC has become well established as the primary immunosuppressant employed by most liver transplant centers. It was proven to be valuable with better graft and patient survival rates post liver transplantation [3]. Therapeutic drug monitoring of TAC in blood is necessary to provide an effective immunosuppression and avoid adverse effects after organ transplantation [4].

Tacrolimus is a metabolic substrate for (CYP450) 3A enzymes – in particular CYP3A5 and is transported out of cells via P-glycoprotein which is the product of ABCB1 gene [5].

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Several studies have suggested a link between the variability in the pharmacokinetics of tacrolimus and the polymorphisms of the CYP3A5 and ABCB1 genes [5]. Tacrolimus has poor bioavailability after oral administration (~25%; range, 4–93%) and is extensively metabolized by the cytochrome P4503A (CYP3A) oxidative enzymes CYP3A4 and CYP3A5 in the liver and small intestine [6]. A single nucleotide polymorphism (SNP) in the CYP3A5 gene involving an adenine (A) to guanine (G) transition at position 6986 within intron 3 was found to be strongly associated with CYP3A5 protein expression. At least one CYP3A5*1 allele was found to express large amounts of CYP3A5 protein, whereas homozygous for the CYP3A5*3 allele did not express significant quantities of CYP3A5 protein, as it causes alternative splicing and results in a truncated protein (which is not functional) and a severe decrease of functional CYP3A5 [4].

TAC is also a substrate of P-glycoprotein (P-gp), one of the ATP-binding cassette transporters that actively transport its substrates out of cells by the driving force of ATP [7]. P-gp is encoded by the multi-drug resistance (MDR1) gene, also known as ABCB1 [8]. Physiologically, P-gp is present in the small intestine, liver, kidney, adrenal gland and pancreas [9]. Efflux activity associated with P-gp, therefore, reduces the intestinal absorption of orally administered drugs while enhancing their biliary excretion through the liver and tubular excretion in the kidney [10]. Substitution of cytosine (C) by thymine (T) in the ABCB1 3435 gene in exon 26 is connected with the change of expression and activity of P-gp. It was observed that patients with genotype of ABCB1 343CC (homozygote CC) showed an expression of P-gp in the duodenum endothelium two times higher compared to individuals with ABCB1 343TT genotype (homozygote TT). However, the intermediate values of P-gp activity and expression were noted in patients with ABCB1 3435CT genotype (heterozygote CT) compared to both homozygote groups [11].

The aim of this pilot study is to assess the influence of ABCB1 (3435 C>T) and CYP3A5 (6986 A>G) genes polymorphisms of both donors and recipients of liver transplant on blood level and dose requirements of oral tacrolimus, in an attempt to help in designing an individualized tacrolimus regimen for Egyptian liver transplant recipients.

2. Subjects and methods

This study was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans. Fifty consecutive adult LT recipients and their living donors were prospectively included in the study (two patients died during the first two weeks post transplantation and were excluded from the study). Recipients underwent graft LT at National Liver Institute (NLI), Menoufia University in the period from January 2011 to July 2014; they were 44 males and 4 females with age ranged from 24 to 51 years. Their donors were 27 males and 21 females with age ranged from 21 to 40 years. The study protocol was approved by the ethics committees of the NLI and Faculty of Medicine, Menoufia University. A written informed consent was taken from all subjects prior to enrollment in this study. Patients were followed up in the Liver Transplantation Unit, NLI during the first postoperative month then in the Hepatology Department till the end of the sixth month (endpoint of the study).

Adult recipients were included if they were given TAC-based immunosuppressive regimen for 6 months. They were excluded if they received any immunosuppressive regimen during the first six months other than TAC, had acute rejection, graft failure or severe post-LT complications necessitating TAC dose modification as severe infections.

Recipients' body weights were recorded and laboratory investigations including blood glucose level, total and direct bilirubin, urea and creatinine were done preoperatively and 1, 3 and 6 months postoperatively, using the Autoanalyzer SYNCHRON CX9ALX (Beckman Coulter Inc., CA, USA).

After transplantation, the recipients were given TAC orally from the first day post-operative. The dose was subsequently adjusted according to the whole blood trough levels where the target trough level was 10–15 ng/ml in the first 4 weeks then 5–10 ng/ml till the end of follow-up according to the followed treatment protocol at NLI.

2.1. Therapeutic drug monitoring of tacrolimus (TDM)

Daily weight adjusted dose of TAC was recorded, the dose was 0.05–0.14 mg/kg/day divided into 2 doses during the first month, was 0.06–0.142 mg/kg/day after the first month and it was 0.08–0.19 mg/kg/day at 3 and 6 months. Measurement of whole blood TAC trough concentration (ng/ml) on 1st, 2nd and 3rd days, after 1 and 2 weeks, and at 1, 3 and 6 months post-operative using ultra performance liquid chromatography tandem mass spectrometry (LC/MS/MS analysis) (ACQUITY UPLC H-Class. Waters Corporation, MA, USA). Whole blood samples were taken at 9 am, 2 h before the next TAC dose. Concentration/dose (C/D) ratio was calculated by dividing TAC trough blood concentration by the corresponding weight adjusted daily dose (mg/kg/day).

2.2. ABCB1 and CYP genes genotyping

Five ml of venous blood were withdrawn from the recipients and their donors, preserved in Ethylenediaminetetraacetic acid (EDTA) containing tubes and stored at -80 °C for genotyping. DNA was extracted from frozen EDTA treated blood sample using Gene JET™ Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Inc. EU/Lithuania).

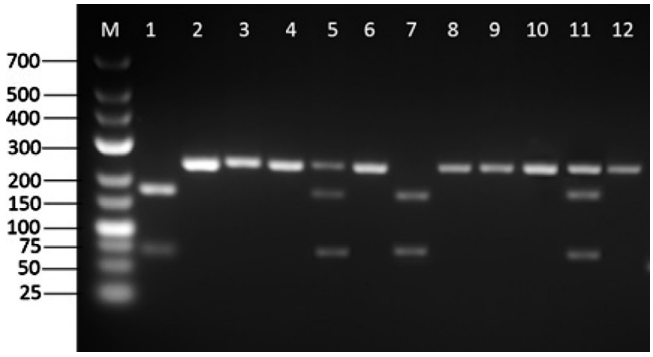
(A) Genotyping of CYP450 3A5 (6986A>G, rs776746) by TaqMan allelic discrimination assay technique: The presence of two primer/probe pairs in each reaction allows genotyping of the two possible variants of a single nucleic acid sequence at the SNP site in a target template sequence. The Allelic Discrimination assay classifies unknown samples as: homozygotes (samples having only allele 1 or allele 3) and heterozygotes (samples having both allele 1 and allele 3).

The primers were: forward (5′-CATCAGTTAGATGACAGATGA-3) and reverse (5′-GGTCCAAACAGGGAAGAAATA-3) (Bioneer, Inc. USA). Probes were: Allele 1 (5′-FAM-GGTGGCTGGGCCGGGGCTGT CCAGT-BHQ-3′) and Allele 3 (5′-HEX GGTCCAAACAGGGAA GAAATA-BHQ-3′) (Bioneer, Inc. USA) [12]. PCR amplification of CYP3A5 was carried out to a total volume of 25 µl, containing 5 µl (0.1 µg/µl) of genomic DNA template, 12.5 µl Maxima probe Master Mix (2X) (Thermo scientific), composed of Maxima Hot Start Taq DNA polymerase and dNTPs in an optimized PCR buffer, 0.3 µl (30 pmol) of each primer, 1.2 µl (12 pmol) of each probe and 4.5 µl of nuclease-free water. Amplification of DNA product was performed by pre-programmed thermal cycler LINE GENE 9660 (BIOER Technology, Co.,LTD, JAPAN), the PCR cycling conditions were initial denaturation for 10 min at 95 °C, followed by 50 cycles with denaturation of 15 sec at 92 °C and 60 s at 60 °C for annealing and extension.

(B) Genotyping of ABCB1 gene (3435C>T, rs1045642) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP): Amplification of DNA product was performed by pre-programmed thermal cycler (Techne, E.U.) using the following primers (Metabion International, Germany): Forward: 5′-GATCTGT GAACTCTTGTTTTCA-3; Reverse: 5′-GAAGAGAGACTTACATTAGGC-3 [12].

PCR amplification of exon 26 of the ABCB1 gene was carried out to a total volume of 26.5 µl, containing approximately 100 ng of genomic DNA (10 µl), 0.5 µl of each 100 pmol/L primers, 12.5 µl of Dream Taq green PCR master mix (2X) (Fermentas, Life Science, E.U.) (containing dream Taq DNA polymerase in 2X Dream Taq green buffer, dATP, dCTP, dGTP, dTTP, 0.4 mM each and 4 mM MgCl2) and 3 µl sterile H_2O. Reaction conditions included initial denaturation at 95 °C for 10 min, followed by 40 cycles, 30 s at 94 °C, 30 s at 54 °C, and 45 s at 72 °C, then a final extension at 72 °C for 5 min. PCR product (244 bp) was digested with the restriction enzyme MboI fast-digest (Fermentas, Life Science, E. U.). 10 µl PCR product, 2 µl 10× buffer, 7 µl nuclease-free water, 1 µl MboI -fast digest, were mixed gently and incubated at 37 °C for 30 min. The reaction mixture was electrophoresed through 3% agarose gel stained with ethidium bromide, 25 bp gene ruler DNA ladder (Thermo Scientific Inc, USA) was used. Fragments of DNA were photographed under ultraviolet transillumination (Syngene, Bioimaging, Ingenius, Canada). Fragments from patients who were homozygous for the SNP (TT) 3435 C>T were uncut and showed a single band at 244 bp and 4 (not seen). Fragments from heterozygotes (CT) were partially digested and showed three bands at 244 bp, 172 bp and 68 bp. Fragments of the wild type allele C were digested and showed two bands that are 172 bp, 68,bp and 4 (not seen) [13].

2.3. Statistical analysis



Gel imaging of ABCB1 genotypes: Lanes 1 and 7 show CC genotype characterized by the presence of 172 bp and 68 bp fragments. Lanes 2, 3, 4, 8, 9, 10 and 12 showed TT genotype recognized by the 244 bp fragment. Genotype CT was found in lanes 5 and 11, where it was identified by the presence of 244 bp, 172 bp and 68 bp fragments. Lane M showed 25 bp gene ruler DNA ladder, with land mark bands at 100 bp and 300 bp.

Results were collected, tabulated, statistically analyzed by IBM personal computer and statistical package SPSS, version 19.0 (IBM Corp, Armonk, NY, USA). The current study showed normal distribution of allelic variants of both ABCB1 and CYP3A5 genes according to Hardy Weinberg test of equilibrium.

3. Results

3.1. Characteristics of study population

A total of 48 consecutive adult LT recipients and their living donors were prospectively included in this study with mean age of 45.8 ± 5.7 (91.7% were males), their 48 donors mean age was 28.8 ± 6.8 (56.3% of them were males). The mean age of donors is lower than recipients as they were selected with younger age to be fit for the operation and give better graft survival results.

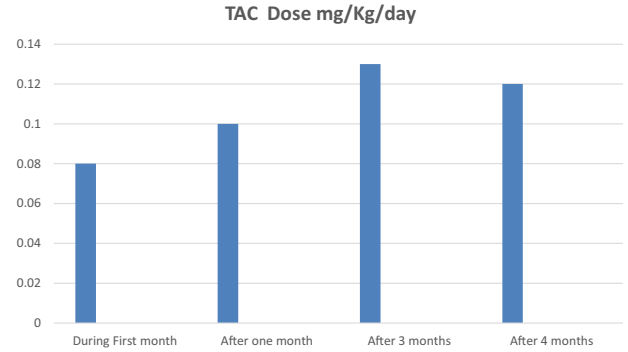


Fig. 1. TAC doses among recipients during the study period.

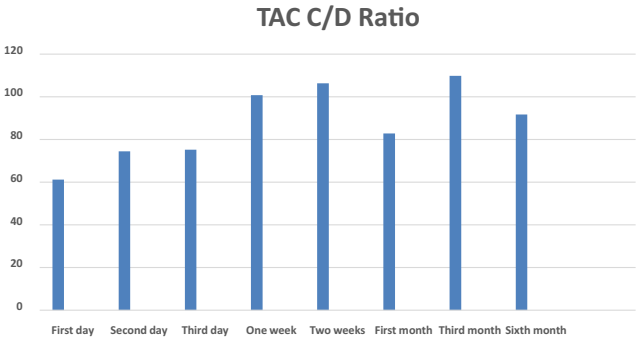


Fig. 2. TAC C/D ratios among recipients during the study period.

Table 1
Distribution of CYP3A5 and ABCB1 genotypes in liver transplant recipients and donors.

Studied genes	Genotypes	Recipients (n = 48) n (%)	Donors (n = 48) n (%)
CYP3A5	*1/*1	10 (20.8)	10 (20.8)
	*1/*3	20 (41.7)	18 (37.5)
	*3/*3	18 (37.5)	20 (41.7)
ABCB1	CC	22 (45.9)	22 (45.9)
	CT	16 (33.3)	16 (33.3)
	TT	10 (20.8)	10 (20.8)

n = number, data are presented as number and percentage.

3.2. Variations of tacrolimus daily dosage and concentration/dose (C/D) ratio during the period of follow up

TAC dose was gradually increased during 6 months post transplantation to reach the desired TAC trough concentration, as during the 1st month the mean dose was 0.08 ± 0.02, and reached 0.10 ± 0.02, 0.13 ± 0.030 and 0.12 ± 0.03 after 1, 3 and 6 months postoperative respectively, while recipients TAC C/D ratios showed two increases during the period of follow up (during the first 2 weeks and 3rd month postoperatively), where the mean C/D ratio of TAC on 1st, 2nd and 3rd days, after 1 and 2 weeks, and at 1, 3 and 6 months postoperative were, 61.19 ± 35.09, 74.42 ± 33.35, 75.19 ± 40.23, 100.74 ± 60.99, 106.27 ± 87.16, 82.78 ± 34.12, 109.82 ± 37.98, 91.68 ± 40.14 respectively. (Figs. 1 and 2), this wide range of tacrolimus daily dose and C/D ratio confirms the large inter individual variation of TAC oral bioavailability.

Frequency of CYP3A5 and ABCB1 genotypes in liver transplant recipients and donors is displayed in Table 1 *where:* CYP3A5*3/*3-genotype was detected in 18 (37.5%), CYP3A5*1/*3 in 20 (41.7%) and CYP3A5*1/*1 in 10 (20.8%) recipients' cases. Their donors genotypes were CYP3A5*3/*3 genotype was detected in 20 (41.7%),

CYP3A5*1/*3 in 18 (37.5%) and CYP3A5*1/*1 in 10 (20.8%) donors, no significant difference was noticed between the two groups ($p > 0.05$).

3.3. For ABCB1 gene

ABCB1 CC genotype was observed in 22 (45.9%), CT in16 (33.3%) and TT 10 (20.8%) recipients. The same distribution was identified in their donors.

3.4. Comparison between different CYP3A5 and between ABCB1 genotypes revealed that: (data are not shown)

No significant difference regarding age, gender, weight, serum levels of bilirubin, urea and creatinine between the different genotypes of both genes, meanwhile significant increase in random blood sugar was observed among ABCB1 CC genotype (wild type, rapid metabolizer) compared to other genotypes; CT and TT preoperatively ($p = 0.008$) and in non- expresser mutant CYP3A5*3/*3 genotype recipients compared to recipients carrying 1*/3* and 1*/1* genotypes at 1 and 6 months postoperatively ($p = 0.02$) and ($p = 0.04$) respectively.

3.5. Effect of CYP3A5 and ABCB1 genes polymorphism on tacrolimus dosage and concentration/dose (C/D) ratio in the liver transplant recipients and donors

3.5.1. For CYP3A5 gene

Over 6 months of follow up post transplantation, TAC daily dose among recipients carrying CYP3A5*3/*3 genotype (non-expressers) were not significantly different from those carrying 1*/3* and 1*/1* genotypes (data not shown). A significant increase (p value < 0.05) in C/D ratios was observed among recipients carrying 3*/3* genotype than recipients carrying 1*/1* and 1*/3* genotypes during 1st, 2nd days and 2 weeks postoperatively, meanwhile no significant difference was observed during the remaining period of follow up. Also, higher C/D ratios (although not statistically significant) were observed in recipients receiving graft with 3*/3* genotype and it reached a statistically significant level only at the end of 1st week postoperatively (p value < 0.05) compared with recipients receiving graft with 1*/1* and 1*/3* genotypes during the period of study (Table 2).

3.5.2. For ABCB1 gene

During and after the first month postoperatively: TAC daily dose was significantly increased among recipients carrying CC genotype (high activity) compared to recipients carrying CT and TT genotypes (data not shown). A significant increase in C/D ratio was detected among recipients carrying CT and TT genotypes compared to those carrying CC genotype (high activity) during the whole period of follow up but; it reached significant trend on 1st, 3rd day and at 3 months postoperatively. Also, a significant increase in C/D ratios was observed in recipients receiving graft with TT and CT genotypes compared to recipients receiving graft with CC genotype late at 3 and 6 months postoperatively; meanwhile no significant difference was observed during the remaining period of the study (Table 3).

To show the impact of the graft with different ABCB1 and CYP3A5 genotypes on the recipients TAC pharmacokinetic activity: The patients were divided into 4 groups regarding each gene:

CYP3A5 Group I: recipients carrying CYP3A5 *1 allele (expressers) receiving graft with CYP3A5 *1 allele; *1/*1, n = 22) (reference group a)
CYP3A5 Group II: recipients carrying CYP3A5 *1 allele (expressers) receiving graft with CYP3A5 *3*/3 non expresser genotype; *1/3*3, n = 8)
CYP3A5 Group III: CYP3A5 *3*/3 non expresser genotype carrying recipients receiving graft with CYP3A5 *3/*3 genotype; 3*3/3*3, n = 12) (reference group b)
CYP3A5 Group IV: CYP3A5 *3/*3 non expresser genotype carrying recipients receiving graft with CYP3A5 *1 allele (expressers); 3*3/*1, n = 6)

Where it was noticed that TAC doses were higher in recipients carrying *1 allele of CYP3A5 gene receiving graft from donors with *1 allele of CYP3A5 gene (group I) compared to recipients carrying *1 allele receiving graft from donors with *3*3 genotype (group II) with significant difference during 1st month. No significant difference was observed in TAC doses between recipients carrying *3/*3 genotype receiving graft from donors with *3/*3 genotype (group III) compared to recipients carrying *3/*3 genotype receiving graft from donors with *1 allele (group IV). A significant decrease was found in TAC C/D ratio at 3rd day and 6th month in recipients carrying *1 allele receiving graft from *1 allele (group I) compared to recipients carrying *1 allele receiving graft from donors with *3*/3 genotype (group II). While TAC C/D ratios were higher in recipients carrying *3/*3 genotype receiving grafts from donors carrying *3/*3 genotype (group III) compared to recipients carrying *3/*3 genotype receiving grafts from donors with *1 allele of CYP3A5 gene (group IV) with significant difference at 1 and 6 months (Table 4).

ABCB1 Group I: recipients with ABCB1 CC genotype (high activity) receiving graft with ABCB1 CC genotype, CC/CC, n = 12 (reference group a)
ABCB1 Group II: recipients with ABCB1 CC genotype (high activity) receiving graft with ABCB1 T allele (low activity), CC/T, n = 10
ABCB1 Group III: ABCB1 T allele carrying recipients (low activity) receiving graft with ABCB1 T allele, CT or TT/CT or TT, n = 16) (reference group b)

Table 2
Tacrolimus C/D post-transplant according to donors' and recipients' CYP3A5 genotype.

C/D ratios (ng/ml)/(mg/kg/day)	Recipient Genotype		P value	Donor Genotype		P value
	*1/*1 and *1/*3 (n = 30) Mean ± SD	*3/*3 (n = 18) Mean ± SD		*1/*1 and *1/*3 (n = 28) Mean ± SD	*3/*3 (n = 20) Mean ± SD	
Day 1	49.7 ± 20.5	82.4 ± 46.2	0.036*	59.5 ± 26.1	62.3 ± 40.4	0.76
Day 2	60.6 ± 21.4	96.7 ± 37.6	0.002*	70.7 ± 25.6	77.1 ± 38.4	0.47
Day 3	64.0 ± 25.5	92.4 ± 52.2	0.09	73.9 ± 20.8	76.8 ± 50.7	0.81
Week 1	45.8 ± 70.0	97.9 ± 12.13	0.186	49.0 ± 14.6	100.5 ± 72.0	0.004*
Week 2	78.08 ± 43.7	156.3 ± 119.3	0.001*	101.9 ± 33.2	109.15 ± 109.9	0.70
Month 1	74.4 ± 29.0	82.7 ± 34.12	0.09	75.0 ± 23.4	88.3 ± 39.5	0.15
Month 3	117.6 ± 43.1	96.7 ± 22.6	0.05	101.0 ± 33.2	116.0 ± 40.4	0.16
Month 6	92.7 ± 47.9	89.7 ± 19.4	0.813	83.1 ± 22.3	98.2 ± 49.1	0.15

SD = standard deviation, C/D = concentration/dose, p value < 0.05 = significant*, the used test: Mann-Whitney Test.

Table 3
Tacrolimus C/D post-transplant according to donors' and recipients' ABCB1 genotype.

C/D ratios (ng/ml)/(mg/kg/day)	Recipients Genotype		P value	Donors Genotype		P value
	CC (n = 22) Mean ± SD	CT and TT(n = 26) Mean ± SD		CC (n = 22) Mean ± SD	Ct and TT (n = 26)Mean ± SD	
Day 1	45.18 ± 24.83	71.88 ± 37.27	0.02*	62.7 ± 39.1	70.7 ± 29.4	0.41
Day 2	77.69 ± 20.02	82.01 ± 39.10	0.19	69.3 ± 41.6	79.0 ± 23.7	0.07
Day 3	46.28 ± 16.68	97.42 ± 38.98	<0.001*	72.1 ± 50.8	77.6 ± 30.7	0.11
Week 1	95.04 ± 73.38	105.57 ± 49.16	0.13	100.7 ± 76.5	98.2 ± 45.4	0.59
Week 2	96.0 ± 48.42	114.34 ± 108.67	0.88	90.3 ± 43.0	126.6 ± 120.7	0.63
Month 1	80.72 ± 34.72	84.53 ± 34.90	0.71	84.9 ± 30.5	81.0 ± 37.4	0.43
Month 3	95.47 ± 41.83	121.96 ± 30.12	0.009*	97.0 ± 31.8	120.7 ± 39.9	0.02*
Month 6	88.30 ± 46.78	94.78 ± 33.66	0.60	73.5 ± 29.4	105.7 ± 42.1	0.01*

SD = standard deviation, C/D = concentration/dose, p value < 0.05 = significant*, the used test: Mann-Whitney test.

Table 4
Tacrolimus blood C/D ratio (ng/ml) / (mg/kg/day) post-transplantation in different groups of combined CYP3A5 genotypes in both donors and recipients.

C/D ratios (ng/ml)/ (mg/kg/day)	Genotype		P value	Genotype		P value
	Group I *1/*1 and *1/*3/*1/*1 and *1/*3 (n = 22) Mean ± SD	Group II *1/*1 and *1/*3/*3/*3 (n = 8) Mean ± SD		Group III *3/*3/*3/*3 (n = 12) Mean ± SD	Group IV *3/*3/1/*1 and *1/*3 (n = 6) Mean ± SD	
Day 1	49.7 ± 20.5	49.7 ± 21.9	0.824	99.9 ± 61.8	69.2 ± 27.7	0.298
Day 2	54.3 ± 23.9	63.4 ± 20.3	0.373	118.1 ± 52.1	83.9 ± 19.1	0.127
Day 3	58.7 ± 28.1	77.4 ± 8.8	0.042*	124.5 ± 76.7	76.4 ± 26.4	0.259
Week 1	68.5 ± 29.5	108.7 ± 77.5	0.347	122.6 ± 36.4	70.6 ± 36.6	0.060
Week 2	73.5 ± 27.3	79.6 ± 48.4	0.727	120.8 ± 21.2	227.3 ± 195.9	0.259
Month 1	67.1 ± 22.3	77.1 ± 31.1	0.452	129.3 ± 42.5	80.2 ± 23.6	0.024*
Month 3	115.6 ± 41.9	118.4 ± 44.5	0.851	91.2 ± 23.1	107.5 ± 19.1	0.259
Month 6	81.4 ± 27.3	106.8 ± 21.4	0.029*	105.9 ± 3.7	84.3 ± 19.6	0.045*

SD = standard deviation, C/D = concentration/dose, p value < 0.05 = significant*, the used test: MannWhitney test.

ABCB1 Group IV: ABCB1T allele carrying recipients (low activity) receiving graft with ABCB1 CC genotype (high activity), CT or TT/CC, n = 10).

TAC dose was higher in recipients with CC genotype (high activity) receiving graft from donors with CC genotype (group I) compared to recipients carrying CC genotype receiving graft from donors with T allele (group II) with significant difference during 1st month (early postoperatively) and at 3, 6 months postoperatively. There was no significant difference in TAC concentration between recipients with CC (high activity) genotype receiving graft from donors carrying CC genotype compared to recipients carrying CC genotype receiving graft from donors with T allele during the study period. There was no significant difference in TAC C/D ratios between recipients with CC (high activity) genotype receiving graft from donors with CC genotype (group I) compared to recipients carrying CC genotype receiving graft from donors with T allele (group II) except at 6 months where there was significant increase in C/D ratio in recipients with CC genotype receiving graft from donors with T allele (group II). There was no significant difference in TAC C/D ratios between recipients carrying T allele of ABCB1 gene receiving graft from donors with T allele (group III) compared to recipients carrying T allele receiving graft from donors with CC (high activity) genotype (group IV) except at 6 months (late postoperatively) where recipients with T allele receiving graft from donors with T allele had significant increase in C/D ratio (Table 5).

4. Discussion

The haplotype distribution of ABCB1 gene in the present study showed that C allele was 62.5% while T allele was 37.5%. Different alleles of ABCB1 gene did not show significant difference between donors and recipients. These frequencies were similar to that of other Caucasian populations (T allele frequency was 40–50%) [14], and different from that documented in Africans and African Americans (T allele frequency was 0.9–13%) [15], and in European Americans (T allele frequency was 62%), Asian Americans (T allele frequency was 27%) and Mexican Americans (T allele frequency was 35%) [16]. In other ethnic groups, like the Africans and African American populations, a lower T frequency has been reported [17].

In the present study the ABCB1 genotypes of the 48 liver transplanted recipients and their donors, were 22 CC (45.9%), 16 CT (33.3%) and 10 TT (20.8%).

Table 5
Tacrolimus blood C/D ratio (ng/ml)/(mg/kg/day) post-transplantation in different groups of combined ABCB1 genotypes in both donors and recipients.

C/D ratios (ng/ml)/ (mg/kg/day)	Genotype		P value	Genotype		P value
	Group I CC/CC (n = 12) Mean ± SD	Group II CC/CT and TT (n = 10) Mean ± SD		Group III CT and TT/CT and (n = 16) Mean ± SD	Group IV CT and TT/CC (n = 10) Mean ± SD	
Day 1	36.2 ± 20.7	59.9 ± 25.5	0.050	75.2 ± 30.6	67.1 ± 46.3	0.197
Day 2	57.12 ± 23.61	71.9 ± 10.8	0.284	83.1 ± 28.1	81.5 ± 52.5	0.411
Day 3	39.09 ± 6.7	53.4 ± 20.6	0.095	105.0 ± 54.6	92.6 ± 26.0	0.833
Week 1	75.46 ± 21.9	111.3 ± 96.1	0.791	112.4 ± 50.8	94.5 ± 46.7	0.205
Week 2	89.4 ± 32.7	101.4 ± 59.4	1.00	156.8 ± 166.8	90.7 ± 48.6	0.773
Month 1	77.1 ± 28.6	83.7 ± 40.1	0.791	86.4 ± 13.9	83.5 ± 42.7	0.291
Month 3	84.4 ± 25.5	108.7 ± 54.1	0.290	128.1 ± 27.3	112.0 ± 33.1	0.205
Month 6	69.7 ± 37.09	110.5 ± 49.13	0.034*	102.6 ± 38.49	79.0 ± 11.1	0.045*

SD = standard deviation, C/D = concentration/dose, p value < 0.05 = significant*, the used test: Mann-Whitney U Test.

Provenzani et al. [18], stated that the CC, CT and TT genotypes in recipients were 9 (17.7%), 26 (51%) and 16 (31.3%) cases, respectively. Among the donors, the C/C, C/T and T/T genotypes were observed in 12 (23.5%), 26 (51%) and 13 (25.5%) cases, respectively.

In the current study, recipients own ABCB1 gene polymorphism affected the TAC daily dose, concentration and C/D ratio during and after the first month early postoperatively. TAC daily dose was significantly increased among recipients carrying CC genotypes with high activity compared to recipients carrying CT and TT genotypes. Where CC genotype carrying recipients needed higher doses to reach the desired concentration early postoperatively (till the beginning of the third month).

A significant decrease of TAC concentration was observed on the third day, first and second weeks postoperatively, in recipients carrying CC genotype (high activity) compared to those carrying CT and TT genotypes, which indicates that T carrying alleles recipients reached trough concentration faster than CC recipients in the first two weeks post operatively.

Significant decrease in C/D ratio was detected among recipients carrying CC genotype (high activity) compared to those carrying CT and TT genotypes early in first and third days post transplantation and after 3 months of follow up which agreed with Liu et al. [19] and Vavadari et al. [20]. However, Shi et al. [21], reported no significant association between ABCB1 polymorphism and TAC pharmacokinetics.

Sakaeda et al. [22], reported that subjects homozygous for the exon 26 3435T variant present a 2-fold reduction of intestinal P-gp, which might influence the TAC bioavailability. Also, Wei-Lin et al. [12], stated that patients with no sequence variant in the exon 26 of the ABCB1 gene are more likely to extrude tacrolimus from enterocytes and therefore need a higher daily dose to achieve adequate blood tacrolimus levels which confirmed the results of the current study.

In an attempt to discover the effect of the donors graft with different ABCB1 genotypes on the recipients' pharmacokinetic activity, recipients were divided into two groups, one with high activity CC genotype and the other with the low activity T allele carrying genotypes. The two groups were further subdivided, where the recipients with the high activity CC genotypes were split up to two groups, the first received a graft with CC genotype homogenous with recipient and the second received T allele carrying graft. Also, recipients with low activity T allele genotypes were split up to two groups, the first received T allele carrying graft homogenous with recipient and the other group took graft with CC genotype.

In this study, the analysis of recipients' and donors' ABCB1 genotypes, revealed that, the weight adjusted doses of TAC were higher in recipients carrying CC genotype of ABCB1 gene and receiving livers from the same genotype compared to those receiving livers with the T allele of ABCB1 during period of the study with significant increase early postoperatively (1st month) ($P = 0.008$) and late after 3, 6 months postoperatively ($P = 0.024$, 0.049 respectively). While recipients carrying T allele and receiving graft from CC genotype donors needed higher TAC doses compared to those receiving T allele grafts late postoperatively at 6 months when the graft became fully functioning. There was no effect of the graft ABCB1 genotype regarding the TAC blood concentration regardless of the recipients' genotype. The ABCB1 genotype of the graft affected TAC C/D ratios at 6 months postoperatively where lower C/D was detected in grafts carrying the CC genotype compared to grafts with T allele regardless of the recipients' ABCB1 genotype as the transplanted liver became fully functioning. These results matched previous results given by Tada et al. [23] and Wei-Lin et al. [12]. These data suggest that ABCB1 polymorphism may be important in liver transplant patients due to their effects on TAC levels in the liver, which might be a good marker to predict the liver graft rejection [24].

In the current study, the haplotype distribution of CYP3A5 gene in the present study showed that *1 allele was 41.7% while *3 allele was 58.3%. Genotypes (*1/*1), (*1/*3) and (*3/*3) of CYP3A5 gene were observed in 20.8%, 41.7% and 37.5% of recipients respectively, while donors showed 20.8%, 37.5% and 41.7% respectively. No significant difference between recipients and donors groups was detected.

Different frequencies were reported by Provenzani et al. [18], who found that CYP3A5*3/*3 genotype was observed in (86.3%) recipient, CYP3A5*1/*3 in (11.7%) and CYP3A5*1/*1 in (2%). For the corresponding donors, CYP3A5*3/*3 was present in (78.4%), CYP3A5*1/*3 in 10 (19.6%) and CYP3A5*1/*1 in (2%).

Differences of genotypes distribution may be attributed to different ethnic groups of the various studies. Where Lamba et al. [25], stated that 90% of Caucasian subjects are homozygous for the non-functional variant CYP3A5*3, the percentage of CYP3A5*3/*3 subjects was 84.9% and the total frequency of the variant *3 was 91.7%. The percentage of CYP3A5*3/*3 subjects is lower (~20–30%) in other ethnic groups, in particular among Africans and African Americans.

It was confirmed that for tacrolimus that reached the liver, the CYP3A5 polymorphism became the key factor. The deficient function of P-450 decoded by CYP3A5*3/*3 genotype made the intrahepatic metabolism efficacy much lower, as it kept the serum tacrolimus concentration at stable targeted levels [12].

In this view, this study showed that there was no effect of the recipients own CYP3A5 polymorphism on mean weight adjusted TAC dose. However, there was a significant increase in TAC blood concentration in the non-expressers *3/*3 genotype who reached the desired blood trough concentration faster during the first two weeks postoperatively, compared to recipients with at least one copy of T allele (*1/*1 and *1/*3) genotypes who considered to express the functioning enzyme, this significant difference was not observed during 1, 3 and 6 months postoperatively. Also, a significant increase was observed in C/D ratio among recipients carrying *3/*3 genotype compared with recipients carrying *1/*1 and *1/*3 genotypes during the first 2 days and at 2 weeks postoperatively, meanwhile no significant difference was observed during the remaining period of the follow up. This may be because in early stages after operation, the conditions of the recipients were not stable, and liver and intestine dysfunction resulting from ischemia reperfusion injury had not recovered [26].

Similar results were given by Gómez-Bravo et al. [27] and Buendia et al. [28]. Another study conducted by De Wildt et al. [29] did not identify any relationship between recipient CYP3A5 genotype and TAC dosing, they supposed that the main reason for this lack of association was probably that variations in TAC deposit are largely dependent on hepatic metabolism and to a lesser extent on intestinal metabolism in the first 14 days after transplantation.

Goto et al. [30] and Day et al. [31] demonstrated that in liver transplant recipients, patients who do not express functional CYP3A5 (individuals homozygous for CYP3A5*3, require significantly less TAC to reach target concentrations compared to patients who do express CYP3A5 (CYP3A5*1 allele carriers, requiring 30–50% higher TAC doses).

Also, Wu et al. [32], stated that individuals with functional polymorphism CYP3A5*3, the 3*3* genotype showed less efficient metabolism of TAC compared with those displaying a wild-type genotype. CYP3A5 *3/*3 genotype displayed sequence variability in intron 3 that caused a cryptic splice site and transcribed an extraordinarily spliced mRNA, and the truncated protein resulted in the absence of normal CYP3A5 protein from tissue.

These results support a possible benefit of a pharmacogenetic dosing strategy, implying the administration of higher TAC initial doses to CYP3A5 expressers to achieve the targeted concentrations more rapidly. This would be promising as achieving optimal drug

exposure in the first days after transplantation is important as this is the period with the highest risk of acute rejection [33,24].

Because most CYP3A5 enzymes are enriched in the intestinal tract and liver, the contribution of the donor liver should be considered when examining the individual variations of tacrolimus pharmacokinetics. In addition, the first-pass elimination of immune suppressants in the gut is thought to account in part for individual variations [34].

In an attempt to find the impact of the donors' graft with different CYP3A5 genotypes on the recipients' pharmacokinetic activity, recipients were divided into two groups, one expresser of protein carrying the *1 allele genotypes and the other with the non- expresser *3*3 genotype. The two groups were further subdivided, where the *1 allele carrying recipients (expressers) were split up to two groups, the first received a graft with *1 allele genotypes homogenous with the recipient and the second received *3*3 genotype graft. Also, recipients with *3*3 genotype were split up to two groups, the first received graft carrying *3*3 genotype homogenous with recipient and the other group took graft with *1 allele genotypes.

In this study, the analysis of recipients' and donors' CYP3A5 genotypes, revealed that, the weight adjusted doses of TAC were higher in recipients carrying *1 allele genotypes of CYPA5 gene and receiving livers carrying the same genotypes compared to those receiving livers with the *3/*3 genotype early postoperatively during the 1st month.

There was no effect of the graft CYP3A5 genotype regarding TAC blood concentration. Recipients carrying *3/*3 genotype (non-expresser) engrafted with CYP3A5 expressers (*1/*1 and *1/*3) achieved lower mean C/D ratio than those with grafts from non-expressers (3*/3*) at the first month. Also, recipients engrafted with 3*/3* genotype had higher C/D at six months regardless of the recipients CYPA5 genotype. These results agreed with other study by Wang et al. [26] who found that higher tacrolimus C/D ratios were detected in CYP3A5 non-expressers in both donors and recipients and concluded that, only the CYP3A5*3/*3 genotype recipients with CYP3A5*3/*3 liver required lower tacrolimus dosage to achieve the target trough concentration.

Buendía et al. [35] found that, dose-adjusted TAC trough concentrations were significantly lower in those in whom the donor or recipient expressed *1 allele compared with those in whom neither the donor nor recipient expressed this allele at 7 days and 2, 3, 6, and 12 months after transplant. Argudo et al. [36] stated that, from the first month after transplantation, patients with grafts from donor carriers of allele*1 had lower concentration-dose ratios compared with patients with grafts from donor non-carriers of that allele. The recipient's genotype for CYP3A5*1/*3-polymorphism had no influence on TAC pharmacokinetics, with no differences between carriers and non-carriers of allele*1.

Tacrolimus pharmacokinetics was influenced by the recipients and donors genotypes which could be explained as, Wei-Lin et al. [12] stated that in the first month after transplantation, early post operatively before the transplanted liver takes over its full function, the absorption functioned before the metabolism started, and P-gp acted as the first clearance effect to tacrolimus, which reduced the serum volume of the drug in vivo, meanwhile later it confirmed that for tacrolimus that reached the liver, the CYP3A5 polymorphism became the key factor. The deficient function of P-450 decoded by CYP3A5*3/*3 genotype made the intrahepatic metabolism efficacy much lower, as it kept the serum tacrolimus concentration at stable targeted levels.

Also, Uesugi et al. [37] and Provenzani et al. [18], proposed that in liver transplant patients, TAC doses needed to reach the target trough blood levels may increase along time. This might be due to the fact that, early after transplantation, the engrafted liver cannot fully perform its metabolic function owing to the damage associated with ischemia and hepatic reperfusion.

5. Conclusion

This study has demonstrated the high variability of the tacrolimus doses required to maintain the desired drug levels. Recipients' ABCB1 genotypes affected the dose requirements and drug blood concentration as well as C/D ratio mainly in the first 3 months post operatively, while the grafts' ABCB1 genotypes mostly affected the drug C/D ratio late mainly at six months postoperatively. Recipients CYP3A5 genotypes affected the drug pharmacokinetics mainly in the first two weeks after transplantation, while the grafts' CYP3A5 genotypes affected the drug C/D ratio late mainly at six months postoperatively after the grafted liver became fully functioning.

Over all in the early postoperative period, in Egyptian liver transplantation patients, the recipients' genetics had a greater impact rather than the donors' genetics on the tacrolimus pharmacokinetics. In the late postoperative period the donors' genetics, rather than that of the recipients', had a more important effect on tacrolimus pharmacokinetics. Thus screening to detect single nucleotide polymorphisms of the above genes prior to transplantation might be helpful for tailoring the appropriate drug regimen for each patient to achieve the desired immunosuppression with minimal side effects.

Declaration of conflict of interest

The authors declare that they have no conflict of interest.

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