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Study of the Effect of CYP3A5 Single Nucleotide Polymorphism on Tacrolimus Metabolism in Liver Transplant Recipients

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ABSTRACT

Background: Tacrolimus is an important immune suppressant medication utilized following transplantation of organ. It is primarily metabolized by cytochrome P450, family 3, subfamily A (CYP3A) enzymes, with significant individual variances in metabolism. **Aim of the work:** This study aimed to examine the effect of the polymorphism of the CYP3A5 rs776746 gene on the blood concentration and tacrolimus dose of recipients and donors, as well as their role in the individualization of tacrolimus dose in Egyptian liver transplant recipients. **Patients and methods:** The investigation comprised 25 liver transplant recipients and their respective donors. Polymorphism of CYP3A5 gene was assessed in all individuals using RT-PCR. For recipients, tacrolimus trough levels were determined, and the weight-adjusted tacrolimus concentration and dosage -to-dose ratio (C/D) have been determined. **Results:** The current investigation demonstrated that recipients with at least one allele of the CYP3A5 3*1 and 1*1 genotypes demanded a larger dosage of tacrolimus compared to those with the 3*3 genotype, but this variance wasn't statistically significant. Recipients of donors with CYP3A5 3*1 and 1*1 genotypes demanded elevated doses of tacrolimus to attain the target trough levels, resulting in a reduced ratio of C/D, in contrast to recipients of donors with the 3*3 genotype, with a statistically significant variance observed during the initial month following transplant.

Conclusion: The investigation found that the CYP3A5 genotype of recipients and grafts demands a greater tacrolimus dose than the 3*3 genotype, and the graft genotype significantly impacts the tacrolimus dose & C/D ratio.

Keywords: Cytochrome P450s, Single nucleotide polymorphism, Liver transplantion.

INTRODUCTION

For more than thirty years, calcineurin inhibitors have been the 1ry immunosuppressive agents in solid organ transplantation [1]. Their introduction marked a significant milestone in the field of organ transplantation [2]. Tacrolimus selectively inhibits calcineurin, consequently impairing the interleukin (IL)-2 transcription & numerous other cytokines in T lymphocytes. Despite its high efficacy in preventing rejection, tacrolimus has a limited therapeutic index & exhibits significant difference in its pharmacokinetics among individuals [3]. The importance of therapeutic drug monitoring (TDM) of tacrolimus is attributed to its narrow target ranges, variability regarding pharmacokinetics, and possibility of medication interaction. Underdosing can result in rejection of graft or even death, while overdosing may result in significant toxicity, like kidney and neurotoxicity. Additionally, the possibility of elevated post-transplant levels of preformed donor-specific antibodies or development of de novo donor-specific antibodies is reduced by achieving an appropriate tacrolimus target [4]. Tacrolimus acts as a metabolic substrate for CYP450. In humans, the most abundant cytochrome proteins are members of the CYP3A subfamily, which are responsible for metabolism of more than fifty percent of medications in use [5]. CYP3A5 is a member of the CYP3A subfamily. It accounts for most of the total cytochrome P450 activity in liver and in the small intestines [6]. Tacrolimus undergoes extensive metabolism by CYP3A5 and is eliminated mainly by the biliary route**.** The P-glycoprotein (P-gp) transporter is responsible for efflux of tacrolimus, which is together with CYP3A5 determines tacrolimus oral clearance. CYP3A5 and P-gp both prevent the absorption of medication into systemic circulation from gastrointestinal tract & aid in its elimination from the body [7].

The pharmacological effects and pharmacokinetics of certain drugs and substances metabolized by the CYP system may be altered by the modulation of CYP expression by cytokines (e.g., TNF-α, IL-6, and IL-1). Furthermore, the expression of CYP genes may be reduced by the release of cytokines [8]. Thus, a complex interplay between the enzymes and cytokines determines tacrolimus blood concentration. Genetic polymorphism in CYP3A5 can result in individual variances in tacrolimus metabolism after liver transplantation by influencing CYP3A5 level and activity [9].

Therefore, this research aimed to study the effect of polymorphism of CYP3A5 rs776746 gene of hepatic transplantation donors and recipients on tacrolimus dose & blood concentration and their role in individualizing tacrolimus dose in Egyptian liver transplant recipient.

PATIENTS AND METHODS

This cross-sectional investigation involved 25 liver transplant recipients and their corresponding donors, recruited from Ain Shams Center for Organ Transplantation (ASCOT).

A- Liver Transplantation recipients (number=twenty-five): This group comprised 25 cases who had undergone current liver transplantation. There were twenty men and

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five women, aged between thirty-six and sixty-seven years.

B- Liver Transplantation donors (number=twenty-five):

Respective donors of liver transplant recipients involved that group. The age of the participants varied from eighteen to forty-one, with eighteen men and seven women.

Exclusion criteria: Subjects who were under eighteen years old, experienced acute rejection or graft failure, or developed complications related to tacrolimus early in the period following transplantation that required an alteration in immunosuppressant protocol.

All individuals in this investigation have been exposed to the following: Complete medical history, clinical assessment & laboratory investigation including total and direct bilirubin, ALT, AST, creatinine, INR, urea, CBC, albumin, and total protein. The real-time polymerase chain reaction (RT-PCR) method was utilized to determine the CYP3A5 rs776746 gene polymorphism of recipients and donors. Tacrolimus concentration/dose ratio (C/D has been calculated by dividing the weight-standardized 24-hour tacrolimus dose (milligram per kilogram per day) by the concentration (nanogram per day). The median of concentration/dose ratio ratios at weeks one to four and the concentration/dose ratio ratios determined at four weeks following transplantation were utilized to quantify weekly alterations in tacrolimus metabolism.

Sampling: Ten milliliters of venous blood have been gathered under complete aseptic conditions two hours prior to the subsequent tacrolimus dose and then separated into four kinds of tubes: A citrate vacutainer for the coagulation profile assay, a plain tube containing gel for serum separation for carrying out chemistry analytes, and two tri-potassium ethylene diamine tetra acetate "K3 EDTA" vacutainer tubes; one for the immediate complete blood count (CBC) assay & tacrolimus level, and the other for real-time polymerase chain reaction. Blood samples in citrate vacutainer were centrifuged at 2500 RPM for 15 minutes and plasma was taken for the assay. Blood has been obtained in plain containers and allowed to coagulate for twenty minutes. Before being centrifuged at 2000-3000 RPM for ten minutes. The liver and renal parameters have been determined by separating the sera. As whole blood, the EDTA vacutainers for PCR have been preserved at -70 ºC until the time of analysis. There was an avoidance of the repeated freezing and thawing of samples.

METHODS

A. Analytical methods

1. **Prothrombin Time** was done on STA- Stago compact C.T. autoanalyzer (Diagnostica Stago,_Inc. Five Century Drive. Parsippany, NJ 07054. United States) using reagents supplied by Neoplastine CI plus (Roche Diagnostics Gmbh, Sandhofer Strasse 116, D-68305 Mannheim).
2. **Blood chemistry assay:** Liver enzymes, total and direct bilirubin, total protein, albumin, creatinine and urea were analyzed on AU 680 chemistry analyzer with dedicated reagents supplied by Beckman Coulter (Beckman Coulter: 250 South Kraemer Boulevard Brea, California USA 92821).
3. **Assay of tacrolimus trough level:** Abbott's competitive immunoassay technique, which utilizes chemiluminescent microparticle immunoassay (CMIA) technology, has been utilized on the Abbott Architect (Abbott: 5050 Nathan Lane North Plymouth, MN USA 55442). The blood specimens have been pre-treated by quickly vortex mixing 200 microliters of EDTA whole blood with two hundred microliters of precipitation reagent, as per the manufacturer's instructions. The resulting clear supernatant was then centrifuged for obtaining it for analysis. There is a competition between tacrolimus acridinium-labeled conjugate and tacrolimus in the specimen for the available binding sites on the paramagnetic microparticles that have been coated with an anti-tacrolimus antibody. The amount of tacrolimus in the specimen is indirectly correlated with the chemiluminescent signal that results.
4. **Assay of CYP3A5 polymorphism by real time PCR:** The TaqMan real-time PCR reagent (Thermo scientific: 168 Third Avenue Waltham, MA United States of America 02451.1) has been utilized to identify the CYP3A5 polymorphism (rs776746). The method has been carried out in three primary stages: An amplification of the DNA that was extracted, an allelic discrimination using real-time PCR, and the extraction of genomic DNA from peripheral blood leucocytes from an EDTA whole blood sample are all examples of the procedures that were performed.
   a. **Principle:** The 5´nuclease assay was utilized in TaqMan genotyping assays for amplifying & identifying specific SNP alleles in purified genomic DNA samples. Two primers were included in each TaqMan genotyping assay to amplify the sequence of interest, & two TaqMan probes were used to identify alleles. The inclusion of two probe pairs in each reaction has the effect of making it easier to genotype the two potential variant alleles that are located at the SNP location in a DNA target sequence. The existence or absence of an SNP is determined by the genotyping assay, which is dependent on an alteration in fluorescence of compounds related to the probes.
   b. **Result interpretation:** A significant raise in the fluorescence of the VIC dye exclusively demonstrated homozygosity for allele 1 (Wild allele), a significant rise in the fluorescence of the FAM dye exclusively demonstrated allele 2 homozygosity (Mutant allele), & a significant rise in the fluorescence of both the FAM and VIC dyes demonstrated heterozygosity of allele 1 & allele 2 (Figure 1).

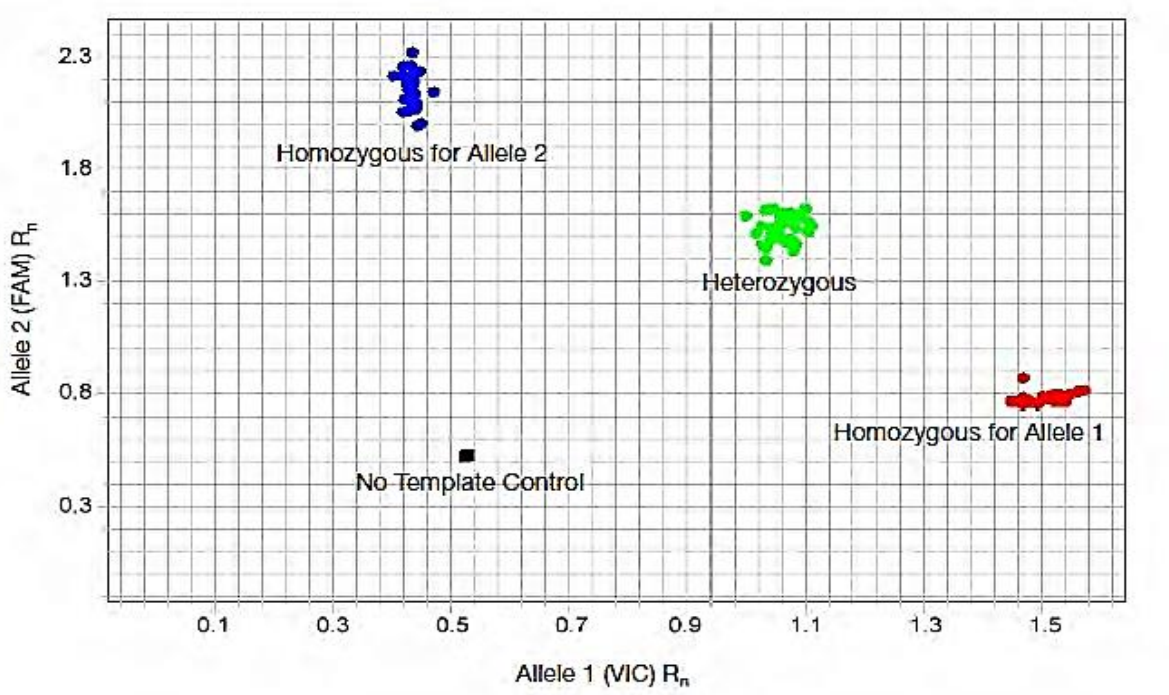

Figure (1): Allelic discrimination plot.

Ethical approval: The Ethics Committee of Ain Shams University, Faculty of Medicine approved this investigation (number MS 364/2019). The patients' data were kept anonymous. Data were presented by diagnosis rather than by patient' name, and patient anonymity was respected. All subjects provided informed permission, which was documented in Arabic and validated by date and time. Confidentiality was ensured by giving a number to the patients' initials, which only the investigator knew. Throughout its implementation, the study complied with the Helsinki Declaration.

Statistical methods

Data were gathered, refined, encoded, & entered into the Statistical Package for Social Science (IBM SPSS) version 23 for analysis. The Friedman test, which is a non-parametric statistical method was utilized to identify variations in therapies over several test attempts. The Chi-square test (X^2) was utilized to examine connection among two variables (Spearman Chi-square) or to compare two independent groups about categorical data. Mann-Whitney U test was utilized for comparing non-parametric data. $P \leq 0.05$ was deemed significant.

RESULTS

Demographic characteristics of the patients and donors: Recipients' group included 80% males and 20% females compared to 72% males and 28% females in the donors' group. The recipients' age ranged from 36 to 67 (54.5 ± 7.5) years while the donors' age ranged from 18 to 41 (30.5 ± 7.5) years.

I. Frequency of CYP3A5 genotype and allele variants in LT recipients and donors: Table (1) showed that for CYP3A5, allele 1 was found to be the minor allele in recipients and donors (8% & 16%), while allele 3 was the major allele with frequency (92% & 84%) in recipients and donors correspondingly. There was an insignificant variance in frequency of CYP3A5 gene polymorphism among recipients and donors.

Table (1): Distribution of CYP3A5 rs776746 genotypes and alleles between donors and recipients

		Recipients n=25 No (%)	Donor n=25 No (%)	X^2	p	
CYP3A5	1*1	1 (4.0%)	3 (12.0%)	1.095	>0.05	NS
	3*1	2 (8.0%)	2 (8.0%)			
	3*3	22 (88%)	20 (80.0%)			
Allele	1	4 (8.0%)	8 (16.0%)	1.515	>0.05	NS
	3	46 (92.0%)	42 (84.0%)			

P-value more than 0.05: Non-significant (NS), P-value not more than 0.05: Significant (S). $X^{\wedge 2}$: Chi square test.

II. Association between CYP3A5 polymorphisms with recipients' C/D ratio, tacrolimus dose and tacrolimus trough level: Table (2) showed comparison between CYP3A5 1 expressors with non expressors in the first 3 months following LT. CYP 3A5 1 expressors (CYP3A5 3*1 and 1*1) showed tendency to greater dose needs of tacrolimus in the 1st two weeks and at second and third month and they had a lower C/D ratio at week 2 & 3 and at 2nd and 3rd month compared to CYP 3A5 non expressors. They also showed lower tacrolimus trough levels at week 3 & 4 and at second month. However, differences were not statistically significant.

Table (2): Effect of recipients′ CYP3A5 genotypes on tacrolimus dose, tacrolimus trough level and concentration/dose ratio during the study intervals (Mann Whitney Test)

	Measured values Median (IQR)	CYP3A5 3*3 n=22	CYP3A5 (3*1) + (1*1) n=3	U	p-value	Sig.
1st week	**C/D**	88.3 (46 – 143.5)	98.8 (44.1 – 140)	-0.084	0.933	NS
	Tacrolimus dose (milligrams per kilogram per day)	0.05 (0.03 – 0.07)	0.06 (0.03 – 0.09)	-0.379	0.705	NS
	Trough level (ng/dl)	4.2 (2.1 – 5.3)	4.3 (4.2 – 5.8)	-0.669	0.503	NS
2nd week	**C/D**	99.35 (66.3 – 137)	75 (66 – 92.5)	-1.046	0.296	NS
	Tacrolimus dose (milligrams per kilogram per day)	0.06 (0.04 – 0.08)	0.08 (0.06 – 0.1)	-1.309	0.191	NS
	Trough level (ng/dl)	5.05 (3.7 – 6.3)	7.4 (4.2 – 8)	-1.004	0.315	NS
3rd week	**C/D**	108.5 (61.6 – 137.5)	84.3 (66 – 114)	-0.418	0.676	NS
	Tacrolimus dose (milligrams per kilogram per day)	0.07 (0.04 – 0.08)	0.05 (0.02 – 0.05)	-1.558	0.119	NS
	Trough level (ng/dl)	5.85 (4.6 – 7.8)	4.5 (3.6 – 5.7)	-1.213	0.225	NS
4th week	**C/D**	117.25 (91.4 – 134.7)	121.4 (75.6 – 220)	0.251	0.802	NS
	Tacrolimus dose (milligrams per kilogram per day)	0.05 (0.04 – 0.07)	0.04 (0.02 – 0.07)	-0.592	0.554	NS
	Trough level (ng/dl)	5.85 (5 – 6.5)	5.3 (3 – 8.5)	-0.126	0.900	NS
2nd month	**C/D**	111.5 (66-184.5)	68.75 (55-82.5)	-1.124	0.261	NS
	Tacrolimus dose (milligrams per kilogram per day)	0.06 (0.04 - 0.1)	0.09 (0.08-0.1)	-1.212	0.225	NS
	Trough level (ng/dl)	6.55 (5.3-8.25)	6.05 (5.5-6.6)	-0.562	0.574	NS

3rd month	**C/D**	119.75 (65.5-150)	108 (99-117)	-0.141	0.888	NS
	Tacrolimus dose (mg/kg/day)	0.05 (0.03-0.08)	0.07 (0.07-0.07)	-0.990	0.322	NS
	Trough level (ng/dl)	4.95 (3.85-7)	7.55 (6.9-8.2)	-1.268	.205	NS

IQR: Interquartile range, C/D: Concentration/Dose

III. Association between CYP3A5 gene polymorphism with donors' C/D ratio, tacrolimus dose and tacrolimus trough level:

As regards CYP3A5 genotypes, table (3) showed that recipients who had their grafts from donors expressing CYP 3A5 1 genotype had lower ratios of C/D throughout the first month and at second month. They also had lower trough levels of tacrolimus and needed higher tacrolimus dose during the same periods. Tacrolimus dose was higher among recipients who received grafts from CYP 3A5 genotype 1 expressors at 1st, 2nd and 4th week and at 2nd month post LT. it was equal at 3rd week and at 3rd month. The difference of the three values was statistically significant at first and fourth weeks post LT.

Table (3): Effect of donors′ CYP3A5 Genotypes on tacrolimus dose, tacrolimus trough level and concentration/dose ratio during the study intervals (Mann Whitney Test)

	Measured values Median (IQR)	CYP3A5 **3*3 n=20**	**(3*1) + (1*1) n=5**	**U**	**p-value**	**Sig.**
1st week	**C/D**	119.75 (60 – 150.75)	39 (34.5 – 70)	-2.140	**0.032**	**S**
	Tacrolimus dose (milligram per kilogram per day)	0.05 (0.03 – 0.06)	0.07 (0.07 – 0.1)	-2.361	**0.018**	**S**
	Trough level (ng/dl)	4.35 (2.5 – 5.55)	2.87 (1.6 – 4.14)	-2.229	**0.026**	**S**
2nd week	**C/D**	100.25 (70.45 – 142.1)	75.5 (66.3 – 92.5)	-1.121	0.262	NS
	Tacrolimus dose (milligram per kilogram per day)	0.06 (0.04 – 0.08)	0.08 (0.06 – 0.08)	-1.063	0.288	NS
	Trough level (ng/dl)	5.1 (4.05 – 6.85)	4.1 (3.7 – 6.8)	-0374	0.709	NS
3rd week	**C/D**	108.5 (66.12 – 141.55)	78 (60 – 112)	-1.121	0.262	NS
	Tacrolimus dose (milligram per kilogram per day)	0.06 (0.04 – 0.08)	0.06 (0.05 – 0.1)	-0.787	0.432	NS
	Trough level (ng/dl)	5.75 (4.05 – 8.4)	5.2 (4.6 – 5.9)	-0.442	0.659	NS
4th week	**C/D**	121.7 (99.45 – 148.35)	91.2 (63 – 99.5)	-1.996	**0.047**	**S**
	Tacrolimus dose (milligram per kilogram per day)	0.05 (0.03 – 0.06)	0.09 (0.05 – 0.2)	-1.993	**0.046**	**S**
	Trough level (ng/dl)	6.1 (6 – 6.1)	5.03 (4.34 - 7)	-2.108	**0.043**	**S**
2nd month	**C/D**	111.5 (66- 184.5)	77.5 (49-106)	-1.124	0.261	NS
	Tacrolimus dose (milligram per kilogram per day)	0.06 (0.04- 0.08)	0.1 (0.1-0.1)	-1.854	0.064	NS
	Trough level (ng/dl)	6.5 (5.1-7.8)	8.25 (5.9-10.6)	-0.492	0.623	NS
3rd month	**C/D**	114.75 (65.5- 147.5)	148 (71- 225)	-0.703	0.482	NS
	Tacrolimus dose(milligram per kilogram per day)	0.05 (0.04- 0.08)	0.05 (0.02- 0.07)	-0.495	0.621	NS
	Trough level(ng/dl)	5.8 (3.85- 7.2)	4.75 (4.5- 5)	-0.564	0.573	NS

IV. Effect of CYP3A5 genotype polymorphisms of the donor on tacrolimus dose, tacrolimus trough level and C/D ratio with corresponding specific recipients' genotype: According to CYP3A5 genotypes, table (4) showed that recipients with CYP3A5 3*3 genotype who received grafts of the CYP 3A5 1 expressors (CYP3A5 3*1 and 1*1) had higher tacrolimus doses, lower trough level -except the fourth week- and lower C/D ratios compared to same recipients who received grafts from donors carrying the CYP3A5 3*3 genotype through almost the whole study intervals. The difference in values of dose of tacrolimus and concentration/dose ratio was statistically significant at first and fourth week. The lower trough level was significant at the end of third month.

Table (4): Effect of donor′s CYP3A5 genotype on dose of tacrolimus, trough level and ratio of concentration/dose when recipient is CYP3A5 3*3 genotype (n=22)

	Measured values Median (IQR)	CYP3A5 3*3(R)- 3*3(D) n=17	CYP3A5 3*3(R) – 3*1/1*1(D) n=5	U	p-value	Sig.
1st	**Tacrolimus dose (milligrams per kilogram)**	0.04 (0.03-0.06)	0.07 (0.07-0.1)	-2.445	**0.014**	**S**
	Trough level (ng/dl)	4.4 (2.4-5.3)	2.87 (1.6-4.14)	-1.333	**0.183**	**NS**
	C/D	126 (72-158)	39 (34.5-70)	-2.116	**0.034**	**S**
2nd week	**Tacrolimus dose (milligrams per kilogram)**	0.05 (0.04-0.07)	0.08 (0.06-0.08)	-1.307	0.191	NS
	Trough level (ng/dl)	5.1 (3.9-6.2)	4.1 (3.7-6.8)	-0.157	0.875	NS
	C/D	113.75 (74.9-147.2)	75.5 (66.3-92.5)	-1.371	0.170	NS
3rd week	**Tacrolimus dose (milligrams per kilogram)**	0.07 (0.04-0.08)	0.06 (0.05-0.1)	-0.552	0.581	NS
	Trough level (ng/dl)	5.9 (4.8-9)	5.2 (4.6-5.9)	-0.823	0.410	NS
	C/D	112 (66.24-145.6)	78 (60-112)	-1.176	0.240	NS
4th week	**Tacrolimus dose (milligrams per kilogram)**	0.05 (0.03-0.06)	0.09 (0.05-0.2)	-1.900	**0.057**	**S**
	Trough level (ng/dl)	5.6 (4.5-6.5)	6.1 (6-6.1)	-0.746	**0.456**	**NS**
	C/D	122.5 (110-162)	91.2 (63-99.5)	-1.998	**0.046**	**S**
2nd month	**Tacrolimus dose (milligrams per kilogram per day)**	0.06 (0.03-0.1)	0.07 (0.04-0.1)	-0.285	0.775	NS
	Trough level (ng/dl)	6.6 (5.9-8.1)	6.4 (5.1-7.95)	-0.328	0.743	NS
	C/D	154 (54-213)	102 (66-141.5)	-0.468	0.640	NS
3rd month	**Tacrolimus dose (milligrams per kilogram per day)**	0.05 (0.04-0.08)	0.07 (0.06-0.07)	-0.997	0.319	NS
	Trough level (ng/dl)	6.5 (5.9-9.2)	5.2 (3.55-6)	-2.124	0.034	**S**
	C/D	112 (68.4-145.6)	101 (63-116.3)	-0.708	0.479	NS

Recipient(R) Donor (D).

DISCUSSION

Tacrolimus is an immunosuppressive agent utilized in organ transplant patients, enhancing case and graft survival rates. Nevertheless, it demonstrates a narrow therapeutic index, resulting in potential rejection or toxicity. Moreover, variability in response from cases need close clinical monitoring and regular therapeutic medication monitoring to ensure both safety and effectiveness [10].

Pharmacogenetics is a novel research horizon that investigates the genetic factors that affect the pharmacokinetics of a medication in the body, as well as the variability of its response. The existence of genetic polymorphisms appears to be associated with this variation. Genotyping is a particularly appealing alternative for the commencement of tacrolimus administration. Additionally, the genotype is a static characteristic that requires only one determination for any given gene, in contrast to phenotypic tests [11, 12].

In this pilot investigation, we investigated the effect of polymorphism of CYP3A5 gene of liver transplant donors & recipients on tacrolimus dose requirement, tacrolimus trough level and C/D ratio. The investigation was carried out on 25 adult cases who underwent transplantation of liver at Ain Shams Center for Organ Transplantation. They were 20 men and 5 women and their 25 relative living donors (18 males and 7 females). The recipients were followed up for 3 months. Pediatric and elderly cases over sixty-five years have been excluded to prevent variation in tacrolimus pharmacokinetics that could be influenced by variations in age [13]. To prevent any potential ethnic factors from influencing tacrolimus levels, the research participants were exclusively Egyptian patients. As demonstrated by **Beermann *et al.*** [14], African American cases needed a greater dose of tacrolimus compared to Caucasians because of significant variances in the absolute bioavailability of tacrolimus between various races.

In this study, CYP3A5 showed predominance of CYP3A5 3*3 genotype among recipients and donors (88% and 80%) respectively. This predominance was observed by other investigators [15, 16] and in a previous Egyptian study done by **Mashaly *et al.*** [17] on kidney transplant patients.

In our investigation, patients received a fixed regimen of steroids and antiproliferative drugs for immunosuppression in addition to tacrolimus. Tacrolimus dose was titrated gradually to reach adequate immunosuppression level. As regards CYP3A5 polymorphism, we detected that recipients expressors of the CYP3A5 *1 allele illustrated an insignificant variance in tacrolimus dose requirement, serum trough level or C/D ratio compared to non expressors, while recipients who received grafts from donors expressors of the minor allele (CYP3A5*1) showed higher dose requirement and lower trough level. This was significant in the first and fourth weeks. The increase in dose requirement reflects increased drug clearance and hence their trough drug level showed lower value. As most of our recipients were CYP3A5 3*3 genotype (88%), we compared them according to the corresponding genotype of the graft received. The five recipients who received grafts from donors expressing the minor allele CYP3A5 3*1 or CYP3A5 1*1 showed constant higher dose requirement and lower trough level and C/D ratio through the whole study duration.

The activity of CYP3A5 has been clarified by **Kuehl and his colleagues** [18], who analyzed liver of human CYP3A5 cDNA and demonstrated that only individuals with at least one CYP3A5*1 allele (A at position 6986) yield an elevated concentration of full-length CYP3A5 mRNA & express CYP3A5 with full metabolic function. CYP3A5 is not expressed in individuals who possess the CYP3A5*3 allele (G at position 6986), which yields a cryptic splice location.

Our outcomes indicated that as regards CYP3A5, the donor graft type significantly affects tacrolimus dose requirement while recipient genotype has no significant effect. In comparison with the donor CYP3A5 non-expressor genotype, a distinct correlation has been found between donor CYP3A5 expressor genotype & a reduced tacrolimus blood concentration/dose ratio in recipients. This can be accounted for by the fact that tacrolimus is primarily dependent on metabolism of liver and to a lesser extent on intestinal metabolism. Additionally, the liver graft has a primary role in the clearance of medications, whereas the gut's CYP3A5 is not the primary factor [19].

When a liver is transplanted, the recipient's old liver is replaced with a new liver that has a genetic background that is distinct from their own. Due to the fact that the majority of the enzymes that are responsible for metabolism of medication are found in the liver, the contribution of the donor liver & the recipient gut on the metabolism of tacrolimus is determined independently by genetic background of both the donor & the recipient. This makes the mechanism of metabolism of tacrolimus extremely complicated [20]. In contrast to other solid organs transplantation; kidney, heart and lung have no effect on tacrolimus metabolism.

Our results agree with **Argudo *et al.*** [16] who stated that recipient genotype for CYP3A5polymorphism doesn't affect pharmacokinetics of tacrolimus and no difference was observed among non-carriers & carriers of the minor allele *1. Nevertheless, some studies showed that recipient CYP3A5 genotypes affect tacrolimus

metabolism. Such as ***Provenzani et al.*** [21] who stated that the existence of at least one *1 copy in the recipient genotype have a tendency to raise tacrolimus doses and to reduced dose-adjusted concentration. However, the variances with respect to the *3/*3 genotypes were statistically insignificant. **Shi *et al.*** [22] discovered that cases with a native CYP3A5*1 allele exhibited a significantly reduced tacrolimus trough concentration & dose-to-concentration ratio than CYP3A5 *3/*3 homozygotes on the seventh day following LT. Another investigation by **Zhu *et al.*** [23] on 95 liver transplant cases detected that tacrolimus clearance increases when patients were CYP3A5 expressor compared to corresponding CYP3A5 non-expressor. The reported recipients' effect on dose requirement and tacrolimus level may be due to early evaluation during the first week so that the graft effect is still not fully apparent or to higher number of studied cases.

However, certain reports indicated that the CYP3A5 polymorphism played a role in both donors and recipients. **Buendía *et al.*** [24] discovered that dose-adjusted tacrolimus trough concentrations were significantly reduced in individuals in whom the donor or recipient expressed one *1 allele in comparison with those in whom neither the donor nor recipient expressed this allele at day 7, 2-, 3-, 6-, and 12-months following transplant. The concentration/dose ratio was additionally found to be higher in the CYP3A5 *3/*3 genotype in both the donor & recipient in the meta-analysis conducted by **Naushad *et al.*** [25] than in the *1/*1 and *1/*3 genotypes. Therefore, the amount of tacrolimus essential to achieve optimal trough levels will be greater if the recipient or donor possesses the CYP3A5*1 allele.

In other words, the tacrolimus C/D ratio is more readily influenced by the existence or absence of expression of CYP3A5 in liver graft compared to the recipient's intestines. According to **Kuehl *et al.*** [18]**,** the hypothesis that the donor CYP3A5 genotype might be more responsible for the significant interindividual variation in dose of tacrolimus required compared to the recipient genotype is partially supported by their findings.

Thus, most investigations supported the theory of the importance of CYP3A5 genotypes in the pharmacokinetics, where tacrolimus concentrations were significantly correlated with the CYP3A5 genotype. Eventually, genotyping for CYP3A5 may facilitate the optimum individualization of immunosuppressive medications treatments for cases having liver transplantation [26].

Hesselink *et al.* [27] stated that the removal of tacrolimus has been clearly influenced by the CYP3A5 genotype in numerous ethnic groups. However, there was not sufficient proof to demonstrate that CYP3A5 genotype-guided tacrolimus administration would improve clinical results. In 2015, the clinical pharmacogenetics implementation consortium (CPIC) published a dosing guideline for tacrolimus that suggests raising the initial dose by 1.5 to 2 times the suggested starting dose in cases who are CYP3A5 intermediate or extensive metabolizers [28].

In the recent work, we investigated tacrolimus pharmacokinetics in a relatively homogenous group of living donor liver transplant recipients (LDLT) recipients. It is essential to highlight that in subjects who required higher doses of tacrolimus throughout the early duration following transplantation, different immunosuppressant drugs as antimetabolites were added or increased to minimize the toxic effect of prolonged high doses of tacrolimus for keeping a normal level of liver enzymes and to avoid rejection.

LIMITATIONS

Limited sample size of the study group may have an impact on the results, which could need further studies on larger sample size and longer monitoring period of immunosuppressant therapy.

CONCLUSION

Our findings suggest that a pharmacogenetic dosing strategy may be advantageous, as they suggest that donor CYP3A5*1 expressors should receive greater tacrolimus initial dosages in order to attain the desired concentrations more quickly. This would be promising, as the initial days following transplantation possess the greatest possibility of acute rejection, which would allow for optimal drug exposure.

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REFERENCES

1. **Lu H, induction Lu L, Zhang F, Zhai Y, Wang X (2016):** Living donor liver transplantation: where do we stand and where are we going? Hepatobiliary surgery and nutrition, 5 (2): 141-144.
2. **Tron C, Lemaitre F, Verstuyft C *et al.* (2019):** Pharmacogenetics of membrane transporters of tacrolimus in solid organ transplantation. Clinical pharmacokinetics, 58 (5): 593-613.
3. **Penninga L, Wettergren A, Wilson C, Chan A, Steinbrüchel D, Gluud C (2014):** Antibody induction versus placebo, no induction, or another type of antibody for liver transplant recipients. Cochrane Database Syst Rev., 5 (6): CD010253.
4. **Mohammadpour N, Elyasi S, Vahdati N, Mohammadpour A, Shamsara J (2011):** A Review on Therapeutic Drug Monitoring of Immunosuppressant Drugs. Iran J Basic Med Sci., 14 (6): 485–498.
5. **Wright W, Chenge J, Chen T (2019):** Structural perspectives of the CYP3A family and their small molecule modulators in drug metabolism. Liver research, 3 (3-4): 132-142.
6. **Kimpton J, Hughes A, MacPhee I (2019):** CYP3A5 genotype had no influence on renal function after renal transplantation. Ethnicity, 58 (31): 184-185.

7. **Grover A, Frassetto L, Benet L, Chakkera H (2011) :** Pharmacokinetic differences corroborate observed low tacrolimus dosage in native American renal transplant patients. Drug metabolism and disposition, 39 (11): 2017-2019.
8. **Stipp M and Acco A (2005**): Involvement of cytochrome P450 enzymes in inflammation and cancer: A Review. Cancer Chemother Pharmacol., 87 (3): 295-309.
9. **Chen S, Li J, Meng F *et al.* (2013):** Individualization of tacrolimus dosage basing on cytochrome P450 3A5 polymorphism–a prospective, randomized, controlled study. Clinical transplantation, 27 (3): E272-E281.
10. **Zhu W, Xue L, Peng H *et al.* (2018):** Tacrolimus population pharmacokinetic models according to CYP3A5/CYP3A4/POR genotypes in Chinese Han renal transplant patients. Pharmacogenomics, 19 (13): 1013-1025.
11. **Chen L, Prasad G (2018):** CYP3A5 polymorphisms in renal transplant recipients: influence on tacrolimus treatment. Pharmacogenomics personalized medicine, 11: 23-33.
12. **Hannachi I, Chadli Z, Kerkeni E *et al.* (2021):** Influence of CYP3A polymorphisms on tacrolimus pharmacokinetics in kidney transplant recipients. The pharmacogenomics journal, 1: 69-77.
13. **Ebid A, Mohamed S, Mira A, Saleh A (2019):** Pharmacokinetics of Tacrolimus in Egyptian Liver Transplant Recipients: Role of the Classic Co-variables. Journal of Advanced Pharmacy Research, 3 (4): 182-193.
14. **Beermann K, Ellis M, Sudan D, Harris M (2014):** Tacrolimus dose requirements in African American and Caucasian kidney transplant recipients on mycophenolate and prednisone. Clin Transplant, 28 (7): 762-767.
15. **Provenzani A, Notarbartolo M, Labbozzetta M (2011):** Influence of CYP3A5 and ABCB1 gene polymorphisms and other factors on tacrolimus dosing in Caucasian liver and kidney transplant patients. International journal of molecular medicine, 28 (6): 1093-1102.
16. **Argudo A, de Aledo J, Alía P (2015):** Liver transplant patient carriers of polymorphism Cyp3a5* 1 donors may need more doses of tacrolimus from the first month after transplantation. In Transplantation proceedings, 47 (8): 2388-2392.
17. **Mashaly M, Mendrinou E, Al Okily A *et al.* (2020):** CYP3A5 Gene-Guided Tacrolimus Treatment of Living-Donor Egyptian Kidney Transplanted Patients. Front. Pharmacol., 11: 1218.
18. **Kuehl P, Zhang J, Lin Y *et al.* (2001):** Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet., 27 (4): 383-391.
19. **Helal M, Obada M, Abd Elrazek W *et al.* (2017):** Effect of ABCB1 (3435C> T) and CYP3A5 (6986A> G) genes polymorphism on tacrolimus concentrations and dosage requirements in liver transplant patients. Egyptian Journal of Medical Human Genetics, 18 (3): 261-268.
20. **Yu S, Wu L, Jin J, Yan S, Jiang G, Xie H, Zheng S. (2006):** Influence of CYP3A5 gene polymorphisms of donor rather than recipient to tacrolimus individual dose requirement in liver transplantation. Transplantation. 81 (1): 46-51.
21. **Provenzani A, Notarbartolo M, Labbozzetta M *et al.* (2009):** The effect of CYP3A5 and ABCB1 single nucleotide polymorphisms on tacrolimus dose requirements in Caucasian liver transplant patients. Ann transplant., 14 (1): 23-31.
22. **Shi Y, Li Y, Tang J, Zhang J *et al.* (2013):** Influence of CYP3A4, CYP3A5 and MDR-1 polymorphisms on tacrolimus pharmacokinetics and early renal dysfunction in liver transplant recipients. Gene, 512 (2): 226-231.
23. **Zhu L, Yang J, Zhang Y, Jing Y, Zhang Y, Li G (2015):** Effects of CYP3A5 genotypes, ABCB1 C3435T and G2677T/A polymorphism on pharmacokinetics of Tacrolimus in Chinese adult liver transplant patients. Xenobiotica, 45 (9): 840-846.
24. **Buendia J, Bramuglia G, Staatz C (2014) :** Effects of combinational CYP3A5 6986A> G polymorphism in graft liver and native intestine on the pharmacokinetics of tacrolimus in liver transplant patients: a meta-analysis. Therapeutic drug monitoring, 36 (4): 442-447.
25. **Naushad S, Pavani A, Rupasree Y *et al.* (2019):** Recipient ABCB1, donor and recipient CYP3A5 genotypes influence tacrolimus pharmacokinetics in liver transplant cases. Pharmacological Report, 71 (3): 385-392.
26. **Shao J, Wang C, Fu P, Chen F, Zhang Y, Wei J (2020):** Impact of Donor and Recipient CYP3A5* 3 Genotype on Tacrolimus Population Pharmacokinetics in Chinese Adult Liver Transplant Recipients. Annals of Pharmacotherapy, 54 (7): 652-661.
27. **Hesselink D, Bouamar R, Elens L *et al.* (2014):** The role of pharmacogenetics in the disposition of and response to tacrolimus in solid organ transplantation. Clinical pharmacokinetics, 53 (2): 123-139.
28. **Birdwell K, Decker B, Barbarino J *et al.* (2015):** Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 Genotype and Tacrolimus Dosing. Clin Pharmacol Ther., 98 (1): 19-24.