EFFECT OF CYP3A4*22, CYP3A5*3 AND ABCB1 C3435T ON TACROLIMUS METABOLISM IN LEBANESE RENAL TRANSPLANT PATIENTS: A RETROSPECTIVE STUDY

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OBJECTIVES

Tacrolimus is one of the major immunosuppressive drugs used after renal transplantation. This drug is metabolized and inactivated by CYP3A4 and CYP3A5. When internalized into the cytoplasm of enterocytes, Pglycoprotein repumps unmetabolized Tacrolimus back into the intracellular medium, which increases the metabolism level.

The aim of this study is to give an idea about the frequency of CYP3A4*22, CYP3A5*3 and ABCB1 C3435T alleles in Lebanese renal transplant patients, and to establish a correlation between these mutations and Tacrolimus metabolism based on Tacrolimus concentration in blood, taking into consideration weight and hemoglobin levels.

METHODS

- 68 whole blood samples were collected from Lebanese kidney transplant patients from SGHUMC.
- DNA extraction was performed using DNA Blood mini Kit-Qiagen according to the manufacturer's protocol.
- End point PCR followed by RFLP were performed to determine the genotypes of CYP3A5 and ABCB1.The restriction enzymes used were MBO1enzyme for ABCB1 and SspI enzyme for CYP3A5.
- As for CYP3A4, genotyping was performed using TaqMan probes on an ABI PRISM 7500 Fast Real Time PCR.

RESULTS

11.8 % of the genotyped sample had at least one CYP3A4*22 allele, 8.8% had at least one CYP3A5*3 allele and 10.3% had 2 wild type ABCB1 C3435T alleles. According to the formula average [tacro]/Daily Dose x weight, the presence of one allele CYP3A4*22 leads to a decrease in tacrolimus metabolism by 1.7 fold. No effect on tacrolimus metabolism in our sample was shown in case of mutations affecting CYP3A5 and ABCB1. The statistical analysis showed that hemoglobin and weight had no effect on blood Tacrolimus concentrations.

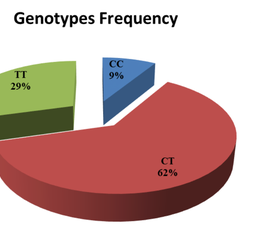

Figure: pie chart representing the percentages of ABCB1 genotypes

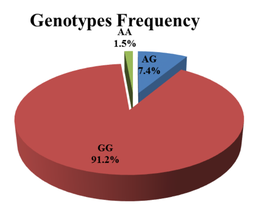

Figure: pie chart representing the percentages of CYP3A5 genotypes

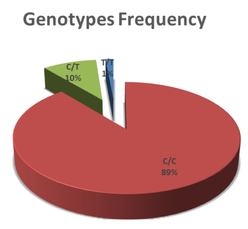

Figure: pie chart representing the percentages of CYP3A4 genotypes

	C/C	C/T + T/T	p-value
First dose	12.60 (±0.58)	9.3 (±1.19)	0.075
Second dose	11.54 (±0.54)	8.00 (±1.36)	0.067
Third dose	10.20 (±0.54)	6.33 (±1.44)	0.075
1st [tacro]/ DD	1.17 (± 0.41)	2.31 (± 1.25)	**0.038**
2nd [tacro]/ DD	1.33 (± 0.71)	2.63 (± 1.35)	**0.023**
3rd [tacro] / DD	1.57 (± 1.13)	3.07 (± 1.73)	**0.040**
1st dose /kg	0.18 (± 0.05)	0.14 (± 0.05)	0.063
2nd dose /kg	0.17 (± 0.05)	0.13 (± 0.06)	0.100
3rd dose/ kg	0.15 (± 0.05)	0.11 (± 0.06)	0.067
Average [tacro]/ DD. kg	1.36 (± 0.73)	2.39 (± 1.50)	**0.026**

Table: Calculations of [Tacro]/DD and dose/kg for the three doses, and of the average[Tacro]/DD.kg depending on CYP3A4 genotypes

Genotype	CC	CT	TT	P-value
First dose	12.40 (± 1.60)	11.74 (± 0.64)	12.40 (± 1.14)	0.899
Second dose	9.60 (± 2.85)	10.76 (± 0.61)	10.80 (± 0.97)	0.919
Third dose	8.80 (± 2.87)	9.39 (± 0.64)	9.50 (± 0.88)	0.912
1st dose/ kg	0.17 (± 0.02)	0.17 (± 0.01)	0.16 (± 0.01)	0.588
2nd dose /kg	0.13 (± 0.04)	0.16 (± 0.01)	0.14 (± 0.01)	0.500
3rd dose/ kg	0.12 (± 0.04)	0.14 (± 0.01)	0.13 (± 0.01)	0.683
1st[tacro] /DD	1.20 (± 0.17)	1.36 (± 0.13)	1.30 (± 0.12)	0.837
2nd [tacro]/ DD	1.48 (± 0.39)	1.52 (± 0.16)	1.38 (± 0.13)	0.953
3rd [tacro] / DD	1.58 (± 0.38)	1.84 (± 0.24)	1.53 (± 0.17)	0.985
Average [tacro]/ DD. kg	0.23 (± 0.04)	0.21 (± 0.01)	0.17 (± 0.01)	0.345

Table: Calculations of [Tacro]/DD and dose/kg for the three doses, and of the average[Tacro]/DD.kg depending on ABCB1 genotypes

Genotype	AA+AG	GG	P-value
First dose	16.40 (± 0.74)	11.69 (± 0.55)	0.070
Second dose	14.80 (± 0.80)	10.50 (± 0.51)	0.058
Third dose	14.40 (± 1.16)	9.03 (± 0.49)	0.190
1st dose/ Kg	0.21 (± 0.016)	0.16 (± 0.007)	0.146
2nd dose /Kg	0.19 (± 0.009)	0.15 (± 0.007)	0.085
3rd dose/ Kg	0.19 (± 0.014)	0.13 (± 0.007)	0.133
1st[tacro] /DD	0.75 (± 0.16)	1.37 (± 0.09)	0.353
2nd [tacro]/ DD	0.75 (± 0.15)	1.53 (± 0.11)	0.228
3rd [tacro] / DD	0.72 (± 0.20)	1.81(± 0.16)	0.304
Average [tacro]/ DD. kg	0.74 (± 0.16)	1.58 (± 0.12)	0.255

Table: Calculations of [Tacro]/DD and dose/kg for the three doses, and of the average[Tacro]/DD.kg depending on CYP3A5 genotypes

	weight	hemoglobin	creatinine
CYP3A4 C/C	70.71 (±2.49)	11.09 (0.28)	1.25 (±0.07)
CYP3A4 C/T+T/T	71.67 (±3.17)	11.71 (±0.84)	1.41 (±0.14)
P-value	0.923	0.366	0.164
CYP3A5 A/A+A/G	72.3 (± 1.16)	14.40 (± 1.16)	1.37 (± 0.09)
CYP3A5 G/G	73.1 (± 3.71)	15.03 (± 0.49)	1.53 (± 0.11)
P-value	0.353	0.190	0.228
ABCB1 C/C	69.18 (± 2.05)	12.14 (± 1.05)	1.15 (± 0.49)
ABCB1 C/T	71.17 (± 3.05)	11.13 (± 0.96)	1.02 (± 0.11)
ABCB1 T/T	72.15 (± 2.05)	12.11 (± 1.16)	0.96 (± 0.16)
P-value	0.345	0.100	0.070

Table: statistical analysis of the significance of weight, hemoglobin and creatinine as potential confounders

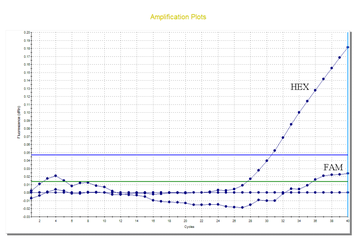

Figure: CYP3A4 TaqMan genotyping results for a C/C patient. Hex dye Corresponds to C, Fam to T

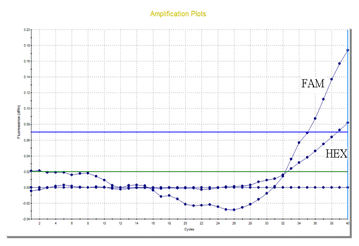

Figure: CYP3A4 TaqMan genotyping results for a C/T patient. Hex dye Corresponds to C, Fam to T

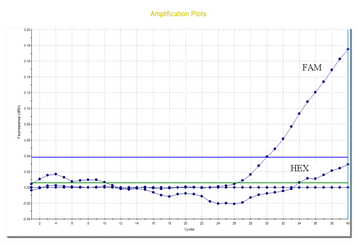

Figure: CYP3A4 TaqMan genotyping results for a T/T patient. Hex dye Corresponds to C, Fam to T

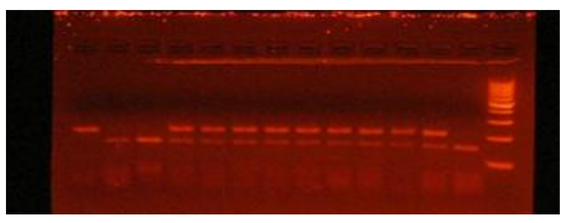

Figure : Gel Electrophoresis Showing RFLP Results of ABCB1 for some Patients using MboI Enzyme. the C/T genotype corresponds to 3 bands of 49, 144 and 193 bp length. The T/T genotype corresponds to 1 band at position 193bp.

Figure : Gel Electrophoresis Showing RFLP Results of CYP3A5 for some Patients using SspI Enzyme. the A/A genotype corresponds to 2 bands at positions 148bp and 168bp. The G/G genotype corresponds to 2 bands at positions 125bp and 168 bp.

CONCLUSION

- The presence of one CYP3A4*22 allele decreases Tacrolimus metabolism, thus increases its bioavailability in blood.
- ABCB1 C3435T and CYP3A5*5 don't have a direct effect on tacrolimus metabolism
- The use of the formula 0.1mg/Kg/12 hrs is not adequate for Lebanese carriers of CYP3A4*22.
- The effects of hemoglobin and weight on Tacrolimus metabolism are negligible in comparison with the effect of the CYP3A4 genotype.
- Diet is a confounder that could be affecting Tacrolimus metabolism.

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