Evaluation of angiotensinogen M235T and T174M polymorphisms, demographic and clinical factors in New-Onset Diabetes after liver transplantation in Iranian patients

Shaghayegh Mottaghi[1], Negar Azarpira[2], Ali Dehshahri[3], Bahman Khalvati[3], Soha Namazi[4]

1-Pharmacotherapy Department, Faculty of Pharmacy, Shiraz University of Medical Science, Shiraz, Iran.
2-Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
3-Biotechnology Department, Faculty of Pharmacy, Shiraz University of Medical Science, Shiraz, Iran.
4-Pharmacotherapy Department, Faculty of pharmacy, Tehran University of medical Sciences, Shiraz, Iran.

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Objectives

- New-onset diabetes after transplantation (NODAT) is a common and serious complication of liver transplantation, which may have a detrimental role in post-transplantation infections, graft rejection and presumably loss.
- Prevalence: In different studies, varies from 2% to 53%.
- Objective: Investigating the effect of two polymorphisms of AGT gene (rs699 named as M235T and rs4762 named as T174M) on NODAT in Iranian liver recipients.

Materials and methods

- 115 patients were collected; 53 NODAT and 62 non-NODAT, all of them underwent liver transplantation between 2007-2013 in Namazi hospital, Shiraz, Iran.
- 80 healthy subjects, never being under transplantation
- Two insertion/deletion polymorphisms (M235T and T174M) of angiotensinogen gene on chromosome1were investigated:
  1.Samples used for patients and control group, were buffy coats and whole bloods, respectively.
  2. DNA was extracted from the samples using QIAamp® DNA Blood Mini Kit.
  3.The DNA was amplified by PCR-RFLP, using Ampliqon® master mix 2 red
  4.PCR products were digested by enzymes Tth111I and NCOI. (respectively for M235T and T174M polymorphisms)
- Some demographic and clinical factors were obtained from patients' files: age at transplantation, sex, BMI, MELD, blood group, prednisolone dose, tacrolimus dose and serum level and mycophenolate mofetil (MMF) dose, all for graft recipients.
- Statistical tests were done in order to determine the effective parameters:
  ◊ Sex and polymorphisms were studied by crosstab test, tacrolimus and mycophenolate mofetil (MMF) dose were investigated by Mann-Whitney
  ◊ ⇨ Age, tacrolimus serum level, Mayo End-stage Liver Disease (MELD) score, BMI, prednisolone dose and pre-transplantation FBS were evaluated by independent samples T test.
  ◊ Allele distribution was evaluated by Hardy-Weinberg using arlequin3.1, with P>0.05 implied as normal distribution.
  ◊ Lastly, a binary logistic regression was conducted among factors showing significant difference between NODAT and non-NODAT.
  Determining the independent risk factor

Results

- Up to 2 years after transplantation, 17.92% (n=266) of all patients got NODAT (among who underwent liver transplantation between 2007 and 2013 in Namazi hospital)
- The most critical time span for NODAT: the first **90** days.
- Pre-transplantation FBS didn't have significant difference between two groups.

Discussion

- According to the table 1, age, prednisolone and mycophenolate mofetil dose and tacrolimus serum level are the demographic and clinical parameters for which a significant difference exists between NODAT and non-NODAT groups. In some previous studies theses factors were shown to have a role in development of diabetes type 2 and NODAT, although some others failed to express any relationship.
- According to the table 2, mutated M235T genotype (MT+TT) may enhance NODAT risk. Genotypes containing wild allele of T174M polymorphism, i.e. MT+TT, may result in NODAT higher risk. There is no previous study investigating angiotensinogen gene and NODAT.
- Mutant allele of M235T may have a detrimental role in NODAT and developing end-stage liver diseases. T allele of T174M can play a role in NODAT development. (Table 3)
- A binary logistic regression showed age, prednisolone dose and M235T polymorphism as independent risk factors. (As seen in table 4)

Table 1. Demographic and clinical information of the liver recipients (N=115)

Demographic and clinical factors		Total patients N=115	Non-NODAT[a] N=62	NODAT N=53	P-value (NODAT vs. non-NODAT)
Age (years), mean±SD[b]		37.39±16.90	30.76±16.87	44.49±13.58	P<0.0001
Sex					
Male, % (n)		40.90% (47)	61.29% (38)	56.60%(30)	P=0.610
Female, %(n)		59.10% (68)	38.71% (24)	43.40% (23)	
BMI[c] (kg/m^2), mean± SD		21.91±5.54	20.81±3.86	22.90±6.65	P=0.238
MELD[d] score, mean± SD		21.17±6.34	20.36±5.77	22.06±6.90	P=0.162
Prednisolone dose(mg/day), mean± SD		8.52±7.29	4.52±5.67	13.21±6.11	P<0.0001
Tacrolimus plasma level (ng/ml), mean±SD		11.12±4.68	8.21±3.06	14.04±4.23	P=0.001
Tacrolimus dose (mg/day), mean±SD		3.45±1.53	3.31±1.19	3.61±1.85	P=0.287
MMF[e] dose (g/day), mean±SD		1.68±0.63	1.55±0.723	1.84±0.56	P=0.021
Blood group % (n)	A	34.80% (40)	35.48% (22)	33.96% (18)	P=0.107
	B	27.80% (32)	35.48% (22)	18.87% (10)	
	AB	8.70% ,(10)	4.84% ,(3)	13.21%,(7)	
	O	28.7% (33)	24.19% ,(15)	33.96%,(18)	
Disease % (n)	Cryptogenic	23.50%, (27)	24.19% ,(15)	22.64% ,(12)	P=0.662
	Cirrhotic	30.40%, (35)	25.81% ,(16)	35.85%, (19)	
	Cholestatic	27.80% ,(32)	29.03% ,(18)	26.41% ,(14)	
	Metabolic	18.30%, (21)	20.97%, (13)	15.10%, (8)	

a. New onset diabetes after transplantation
b. Standard deviation
c. Body mass index
d. Model for end-stage liver disease
e. Mycophenolate mofetil

Table 2. A comparison of M235T and T174M gene polymorphisms in liver recipients and general population

Genotype			General population (n=80)	Total patients (n=115)	NODAT[a] (n=53)	Non-NODAT (n=62)	Odds ratio	P- value
M235T	Wild (MM), n(%)		52(65.00%)	64(55.65%)	18(33.96%)	46 (74.19%)		P[b]<0.0001 P[c]= 0.191
	Mutant (MT+TT), n(%)		28(35.00%)	51(44.35%)	35 (66.04%)	16(25.81%)		
T174M	Co-dominant Alleles N (%)	TT	55 (68.75%)	82 (71.31%)	42 (79.24%)	40 (64.52%)	OR[b]=0.48 OR[c]=1.13	P[b]:0.08 P[c]:0.70
		TM	16 (20.00%)	20 (17.39%)	9 (16.98%)	11 (17.74%)	OR[b]=1.05 OR[c]=0.84	P[b]:0.91 P[c]:0.64
		MM	9 (11.25%)	13 (11.30%)	2 (3.77%)	11 (17.40%)	OR[b]=5.50 OR[c]=1.01	P[b]:0.01 P[c]:0.99
	T recessive N (%)	MM + MT	25 (31.25%)	33 (28.69%)	11 (20.75%)	22 (35.48%)	OR[b]=2.10 OR[c]=0.89	P[b]:0.08 P[c]:0.70
		TT	55 (68.75%)	82 (71.30%)	42 (79.25%)	40 (64.52%)		
	T dominant N (%)	TT + MT	71 (88.75%)	102 (88.69%)	51 (96.23%)	51 (82.26%)	OR[b]=0.018 OR[c]=0.99	P[b]:0.01 P[c]:0.99
		MM	9 (11.25%)	13 (11.31%)	2 (3.77%)	11 (17.74%)		

a. New-Onset Diabetes after Transplantation
b. Comparison between NODAT and non-NODAT
c. Comparison between general population and total patients

Table 3. A comparison of M235T gene polymorphisms in liver recipients and general population

SNP gene	alleles	NODAT[a] % (n=106 alleles)	Non-NODAT % (n=124 alleles)	Total patients % (n=230 alleles)	General population % (n=160 alleles)	P value	OR	Confidence Interval
AGT rs699 (M235T)	M	33.96% (n=36)	74.19 % (n=92)	55.65% (n=128)	70.62% (n=113)	P[b]<0.0001 P[c]=0.003	OR[b]=5.590 OR[c]=1.916	CI[b]:3.166-9.872 CI[c]:1.248-2.940
	T	66.04% (n=70)	25.81 % (n=32)	44.35% (n=102)	29.37% (n=47)			
AGT rs4762 (T174M)	T	87.73% (n=93)	73.39 % (n=91)	80.00% (n=184)	78.75% (n=126)	P[b]= 0.007 P[c]=0.764	OR[b]=2.594 OR[c]=1.079	CI[b]:1.283-5.244 CI[c]:0.656-1.776

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Table 4. Binary logistic regression among factors supposed to be effective on NODAT in univariate tests

parameter	B	Sig.	Odds Ratio	95%Confidence Interval	
				lower	upper
Age	0.059	**0.005**	1.061	1.081	1.106
Prednisolone dose	0.263	**<0.0001**	1.301	1.165	1.451
MMF dose	-0.65	0.198	0.522	0.194	1.404
M235T (1)	1.991	**0.003**	7.326	2.002	26.805
T174M		**0.049**			
T174M(1)	-3.355	**0.037**	0.035	0.001	0.813
T174M(2)	-1.435	0.095	0.238	0.044	1.281

M235T(1): Comparison of MM genotypes with MT+TT in non-diabetics than diabetics
T174M(1): Comparison of MM genotypes with TT in non-NODAT patients rather than diabetics.
T174M(2): Comparison of MT genotypes with TT in non-NODAT patients in non-NODAT patients rather than diabetics.

Conclusion

⇒ **Age**, **prednisolone dose** and **M235T mutated** genotypes may **enhance** NODAT risk in Iranian patients with liver transplantation.

⇒ **T** allele of M235T and T174M may **increase** NODAT risk. The former one, may augment end-stage liver disease risk.

Presenter info:
Prof. Soha Namazi
Email: Snamazi@sums.ac.ir
Mobile: +98 912 738 8655