Evaluation of Angiotensinogen M235T and T174M Polymorphisms, Demographic and Clinical Factors in New-Onset Diabetes after Liver Transplantation in Iranian Patients

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ABSTRACT

Background: New-onset diabetes after transplantation (NODAT) is a serious complication which runs the risk of infections, morbidity and mortality.

Objective: To evaluate M235T and T174M polymorphisms of angiotensinogen gene along with some demographic and clinical factors including age; sex; body mass index (BMI); model for end-stage liver disease (MELD) score; prednisolone, mycophenolate mofetil and tacrolimus dose; and serum level in NODAT among liver recipients.

Methods: In this study 115 patients (53 with and 62 without NODAT) who had no history of diabetes before the transplantation were investigated. Furthermore, 80 randomly selected apparently healthy people (no transplantation) were used as the control group. Two angiotensinogen polymorphisms (M235T and T174M) were studied using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

Results: Patients included 68 (59.1%) females and 47 (40.9%) males; they had a mean±SD age of 37.4±16.9 years. The M allele frequency was 55.7% (n=128) in M235T and 20.0% (n=46) in T174M polymorphisms. Binary logistic regression analysis confirmed that age (p=0.005), prednisolone dose (p<0.001) and mutated M235T polymorphism (p=0.003) were independent risk factors.

Conclusion: Presence of M235T T allele may significantly (p<0.001) increase the NODAT risk, and increase the likelihood of developing end-stage liver disease (p=0.003). T174M T allele had a significantly (p=0.007) higher frequency in NODAT group.

KEYWORD: Liver; Transplantation; Diabetes mellitus; M235T; T174M; Angiotensinogen

INTRODUCTION

New-onset diabetes after transplantation (NODAT) is a common and serious complication of liver transplantation. Since there are some uncertainties about its onset criteria, ranging from 3 to 60 months, the prevalence reported in various studies varies from 0% to 61% [1, 2].

NODAT is a type of diabetes in which patients have a fasting blood sugar (FBS) of <126 mg/dL before transplantation without taking any anti-diabetic medications; FBS, however, increases to ≥126 mg/dL (measured at two occasions) after transplantation. Experiencing polyuria or polydipsia along with a random plasma glucose level of ≥200 mg/dL or a 2-hour glucose tolerance test ≥200 mg/dL,

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or taking any anti-diabetic medications after transplantation, are other clues for making the diagnosis [3, 4]. The pathophysiology of NODAT is similar to type II diabetes mellitus, i.e., insulin-resistance and low secretion [5].

NODAT may have a detrimental role in post-transplantation infections, graft rejection and presumably loss. Therefore, it is vital to identify the impact of risk factors with a purpose of enhancing graft survival and improving patients' life quality [1]. Some risk factors, especially after liver transplantation include recipient's older age [6], ethnicity (most black and Hispanic) [7], pre- and early post-transplantation hypomagnesemia [8], male sex [9, 10], history of diabetes in close relatives [9, 11], immunosuppressive medications including tacrolimus and corticosteroids particularly bolus injection [9, 12], positive hepatitis C serology [8, 9, 13], metabolic syndrome (hypertriglyceridemia, low HDL-C, hyperuricemia, and hypertension) [14], pre-transplantation alcoholic cirrhosis [9], and high body mass index (BMI) [2, 9, 13]. In addition to demographic and environmental factors, genetics can also affect development of NODAT. One study showed that there is no significant relationship between transcription factor-7-like-2 (TCF7L2) rs7903146 polymorphisms and risk of new-onset diabetes after liver transplantation in Iranian population [11].

Parvizi, *et. al.* [12], believe that NODAT is affected by KK genotype in KCNJ11 rs5219 polymorphism, donor and recipient age, male sex, pre-transplantation FBS, and prednisolone dosage in Iranian patients. Lee, *et. al.* [3], studied the influence of angiotensin-converting-enzyme (ACE) gene (rs4291) and angiotensinogen (AGT) gene (rs4291, rs4762) polymorphisms on new-onset diabetes after renal transplantation in a Korean population. It was shown that variants of rs4762 have a determinant role in the incidence of NODAT. Renin-angiotensin-aldosterone system (RAAS) is a major hormonal system regulating blood pressure and balancing fluid and sodium [15]. Hypertension is a state of insulin-resistance, mainly limited to glucose metabolism rather than lipid or potassium [16]. Furthermore, Ang II is suggested to have a role in inhibiting insulin signaling [17], as some evidence states that ACEIs and ARBs may decrease diabetes's risk [18].

There are some explanations for the relationship between NODAT and plasma AGT level and also gene variants. Some genotypes may increase angiotensin II (Ang II) level inappropriately that interferes with glucose transport, preventing insulin signaling [3]. Another insight is that Ang II induces the release of various CC chemokines, such as chemokine ligand 5 (CCL5) to enhance mononuclear leukocyte interaction at arterioles and venules. CCL5 genotype is proved to be linked to NODAT [3, 19], but it is not defined whether there is any association between CCL5 polymorphisms and any components of RAAS, including AGT, genotypes [3].

To the best of our knowledge, the effect of angiotensinogen polymorphisms in post-transplantation diabetes has never been studied in liver recipients in the literature. We conducted this study to investigate the effect of two polymorphisms of AGT gene (rs699 also named M235T, and rs4762 also called T174M) on development of NODAT in Iranian liver recipients.

MATERIALS AND METHODS

Patient Collection

Data were collected from 115 patients who underwent liver transplantation in 2007–2013 in Namazi Hospital, a referral center for organ transplantation in Iran, affiliated to Shiraz University of Medical Sciences (SUMS). Moreover, 80 apparently healthy subjects (not transplanted) were enrolled as the control group. The samples studied from patients and controls included buffy coat taken from sample bank at Transplant Research Center affiliated to SUMS, and whole blood taken from Fars Blood Transfusion Organization, respectively. Clinical and demographic data from patients' files were analyzed too. This study was approved by the SUMS Ethics Committee.

Table 1: Diseases included in each group

	Cryptogenic	Hepatic cirrhosis	Cholestatic	Metabolic
Diseases	Cryptogenic cirrhosis	Hepatitis B	Primary sclerosing cholangitis	Wilson
		Hepatitis C		Hypercholesterolemia
		Hepatitis B+		Tyrosinemia
		Hepatitis C		Progressive familial intrahepatic cholestasis
		Hepatitis C+		
	Non-alcoholic steatohepatitis	Hepatocellular carcinoma	Primary biliary cirrhosis	Hemochromatosis
		HBs Ag+		Biliary atresia
		HBc Ag+		Caroli disease
		Autoimmune Hepatitis		Hyperoxaluria
				Crigler Najjar

Patients were divided into two groups—53 with and 62 without NODAT. In addition to the criteria mentioned above [3, 4] those with a HbA1c >6.9% were considered to have NODAT [4].

The inclusion criteria included all patients who had undergone liver transplantation and had not been diabetic before the procedure or used any anti-diabetic drugs. Those who developed diabetes up to two years after transplantation were considered to have NODAT. The non-NODAT group remained non-diabetic after liver transplantation within the follow-up period mentioned. Those with a diagnosis of diabetes before transplantation were excluded from the study. Also, patients with hypertension (or used anti-hypertensive medications, particularly ACE inhibitors or angiotensin receptor blockers), those who had undergone combined transplantations or other types of transplantation, and patients with incomplete file data, were excluded. Morbidities leading to liver transplantation were classified into four groups in terms of causes of liver failure, as cryptogenic, cirrhosis, cholestatic, and metabolic [20] (Table 1).

DNA Extraction

DNA was extracted using QIAampR DNA Blood Mini kit, 50 preparations. Extraction was done according to the kit's protocol [21]. Absorbance of DNA was measured with Picodrop to examine its quantity and quality. Samples with A260/A280 ≥1.8 (being pure or little contaminated with protein) were considered appropriate for analysis [22].

DNA Amplification

In this study two insertion/deletion polymorphisms (M235T and T174M) of angiotensinogen gene on chromosome 1 were studied by means of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Two 50-µL mixes were made; each contained 1 µL forward and 1 µL of reverse primers (both from the 100 µM stock), and 25 µl of AmpliqonR master mix 2 red; it was added to 10-µL template. According to the authors' experiences, consuming these amounts led to proper results. The primer sequences for each polymorphism studied are [23, 24]:

M235T:

Forward (sense): 5'-CCG TTT GTG CAG GGC CTG GCT CTC T-3'

Reverse (anti-sense): 5'-CAG GGT GCT GTC CAC ACT GGA CCC C-3'

The underlined part of the primer had been modified according to the Tth111I cleavage site so that the enzyme could digest the PCR product.

T174M:

Forward (sense): 5'-TGG CAC CCT GGC CTC TCT CTA TCT-3'

Table 2: Demographic and clinical information of the liver transplant recipients (n=115). Values are either mean±SD or n (%).

Demographic and clinical factors	Total patients (n=115)	Non-NODAT patients (n=62)	NODAT patients (n=53)	p value (NODAT *vs.* non-NODAT)
Age (yrs)	37.4±16.9	30.8±16.9	44.5±13.6	<0.001
Sex				
Male	47 (40.9)	38 (61)	30 (57)	0.610
Female	68 (59.1)	24 (39)	23 (43)	
BMI (kg/m^2)	21.9±5.5	20.8±3.9	22.9±6.7	0.238
MELD score	21.2±6.3	20.4±5.8	22.1±6.9	0.162
Prednisolone dose (mg/day)	8.5±7.3	4.5±5.7	13.2±6.1	<0.001
Tacrolimus plasma level (ng/mL)	11.12±4.68	8.21±3.06	14.04±4.23	0.001
Tacrolimus dose (mg/day)	3.5±1.5	3.3±1.2	3.6±1.9	0.287
MMF dose (g/day)	1.68±0.63	1.55±0.723	1.84±0.56	0.021
Blood Group				
A	40 (34.8)	22 (35)	18 (34)	0.107
B	32 (27.8)	22 (35)	10 (19)	
AB	10 (8.7)	3 (5)	7 (13)	
O	33 (28.7)	15 (24)	18 (34)	
Disease				
Cryptogenic	27 (23.5)	15 (24)	12 (22)	0.662
Cirrhotic	35 (30.4)	16 (26)	19 (36)	
Cholestatic	32 (27.8)	18 (29)	14 (26)	
Metabolic	21 (18.3)	13 (21)	8 (15)	

Reverse (anti-sense): 5'-CAG CCT GCA TGA ACC TGT CAA TCT-3'

The reaction included a preliminary 5 min heating at 95 °C for fully denaturation [25], followed by 45 sec heating at 94 °C for denaturation, 1 min at 65 °C for annealing and getting the primers adhere to the strands, and at the end 1 min at 72 °C for the optimum function of Taq polymerase. Steps 2 to 4 were repeated for 40 cycles. At last, samples were heated at 72 °C for 5 min [23, 24].

The product of the procedure for M235T has a length of 165 base pairs (bp); that for T174M is 353 bp [23].

Mutation Detection

To detect the mutations, a restriction enzyme was added to PCR product to cut DNA at some definite sites near its recognition sequence. Therefore, the shorter created fragments could be observed as distinct bands on electrophoresis gel and helped to differentiate between the genotypes [26].

To detect M235T insertion/deletion mutation, DNA was digested with Tth111I. It broke

Table 3: Comparison of M235T gene polymorphisms frequency between liver transplant recipients and the general population

Gene polymorphism	Genotype	General population, (n=80)	Total patients, (n=115)	NODAT (n=53)	non-NODAT, (n=62)	p value
M235T	Wild (MM)	52 (65%)	64 (55.7%)	18 (34%)	46 (74%)	0.191*
	Mutant (MT+TT)	28 (35%)	51 (44.4%)	35 (66%)	16 (26%)	<0.001†

*Difference between M235T genotypes of general population and total patients

†Difference between M235T genotypes of NODAT and non-NODAT patients

DNA into two distinct fragments, provided that thymine had been substituted with cytosine at the position 704, i.e., locating of threonine amino acid instead of methionine, in the position number 235 of the resultant protein [27]): GACN↓NNGTC [23, 24, 28]. M235T, normal individual M235M, and T235T variants gave three fragments (165-, 141-, and 24-bp), an undigested 165-bp fragment and a 141-bp fragment, respectively [23, 24].

For detecting T174M insertion/deletion mutation of AGT gene, NCOI enzyme was used to break DNA double strand into two 198- and 155-bp fragments if there was a thymine-containing nucleotide in the position number 520 (C↓CATGG) [23, 29]. In this case, threonine had been replaced with methionine [30]. If the sister chromatids showed normal T174, just an uncleaved 353-bp band was obtained after gel electrophoresis. The bands mentioned above appeared after doing electrophoresis on 3% agarose gel in 0.625% TAE buffer environment, and could be observed under UV. Conducting gel electrophoresis on heterozygous samples, led to three bands: an undigested and two digested products [31], although the smallest band might not be observed easily most of the times.

Statistical Analysis

SPSS® for Windows® ver 19.0 was used for data analysis. Quantitative and qualitative data are reported as mean±SD and percent frequencies, respectively. Normality of distribution was examined with one-sample Kolmogorov-Smirnov test. Depending on equality or inequality of variances, independent samples Student's t test or Mann-Whitney U test was used, respectively. Sex and polymorphisms were studied by χ^2 test, tacrolimus and mycophenolate mofetil (MMF) dose were studied by Mann-Whitney U test; age, serum tacrolimus level, Model for End-stage Liver Disease (MELD) score, BMI, prednisolone dose, and pre-transplantation FBS were evaluated by independent samples Student's t test.

A logistic regression analysis was conducted on factors found statistically significant in univariate analysis. Although serum tacrolimus level had been found significant in these tests, it was eliminated from the model, as there was too many missing values.

There were only three patients with heterozygous M235T genotype. Therefore, the groups of two types of mutations were merged. The study power was determined with Power SCC.

RESULTS

There were 1487 patients who underwent liver transplantation between 2007 and 2013 in Namazi Hospital. NODAT developed in 266 (17.9%) patients within two years post-transplantation. Only 115 patients were enrolled in this study according to the inclusion and exclusion criteria. In NODAT group, 42 (79%) developed the disease within the first 90 days.

NODAT and non-NODAT groups were similar in terms of pre-transplantation FBS (93.2±15.9 and 87.6±15.9 mg/dL, respectively; p=0.063). Nor did the groups differ for pre-transplantation HbA1c level (5.4%±0.6% and 5.2%± 0.8%, respectively; p=0.093).

There is a significant difference between the mean age of participants, tacrolimus level, and MMF and prednisolone dosage in two groups (Table 2). Results of two AGT polymorphisms, M235T and T174M, are presented in Tables 3 and 4, respectively. M235T and T174M allele frequencies are presented in Table 5. Binary logistic regression analysis revealed that age, prednisolone dosage, and M235T polymorphism, were independent predictors of NODAT (Table 6).

This study power ranged from 87% to 100% for alleles, and 78% to 100% for genotypes.

DISCUSSION

NODAT incidence ranged from 11% to 61% after liver transplantation [2]. A systematic review and meta-analysis reached an incidence of 13.4% NODAT after solid organ transplantation [1]. Korean kidney recipients showed an

Table 4: Comparison of T174M gene polymorphisms between liver transplant recipients and the general population

Genotype		General population (n=80)	Total patients (n=115)	NODAT (n=53)	Non-NODAT (n=62)	OR (95% CI)
Co-dominant Alleles N(%)	TT	55 (69%)	82 (71.3%)	42 (79%)	40 (65%)	0.48 (0.19-1.19)[b] 1.13 (0.58-2.20)[c]
	TM	16 (20%)	20 (17.39%)	9 (16.98%)	11 (17.74%)	1.05 (0.36-3.08)[b] 0.84 (0.38-1.86)[c]
	MM	9 (11.25%)	13 (11.30%)	2 (3.77%)	11 (17.4%)	5.50 (1.07-37.9)[b] 1.01 (0.38-2.72)[c]
T recessive N(%)	MM+MT	25 (31.25%)	33 (28. 69%)	11 (20.75%)	22 (35.48%)	2.10 (0.84-5.33)[b] 0.89 (0.45-1.93)[c]
	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%)	0.018 (0.83-0.94)[b] 0.99 (0.37-2.66)[c]
	MM	9 (11.25%)	13 (11.31%)	2 (3.77%)	11 (17.74%)	

New-Onset Diabetes after Transplantation

Pearson Chi-square, odds ratio and confidence interval for comparison of alleles between NODAT and non-NODAT

Pearson Chi-square, odds ratio and confidence interval for comparison of alleles between general population and total patients

incidence of 16.2% after a mean follow up of 88 months [3]. A multi-center study reported an incidence of 22.7% among French liver recipients within a mean±SD post-transplant follow-up of 1.8±2.1 months [13]. In our research, this rate was 17.9% after two years of follow-up.

Many studies have so far investigated the risk factors of NODAT [10, 14, 32]. However, there is no consensus on almost none of the identified factors. Age, sex, BMI, MELD score, prednisolone, tacrolimus level, tacrolimus and MMF dosage are non-genetic factors examined in this research. However, the main focus of this study was on two mutations of AGT gene, M235T (rs699) and T174M (rs4762), which have not been studied in post-liver transplantation yet.

In this study, age was found a risk factor for NODAT. Those who developed NODAT were significantly older than those who did not. Logistic regression analysis showed that for each year increase in age the likelihood of developing NODAT increased by 1.06 fold (95%

Table 5: Frequency distribution of alleles in M235T and T174M polymorphisms in the general population and liver transplant recipients

SNP Gene alleles		NODATa, (n) %, (106 alleles)	Non-NODAT, (n) %, (124 alleles)	Total patients, (n) %, (230 alleles)	General population, (n) %, (160 alleles)	OR (95% CI)
AGT rs699 (M235T)	M	36 (34.0)	92 (74.1)	55.65% (n=128)	113(70.6)	5.590 (3.166-9.872)[b]
	T	70(66.0)	32 (25.8)	102 (44.4)	47 (29.4)	1.916 (1.248-2.940)[c]
AGT rs4762 (T174M)	T	87.73% (n=93)	73.39 % (n=91)	80.00% (n=184)	78.75% (n=126)	2.594(1.283-5.244)[b]
	M	13 (12.3)	33(26.6)	46(20.0)	34(21.3)	1.079 (0.656-1.776)[c]

New-Onset Diabetes Mellitus

Pearson Chi- square, odds ratio and confidence interval for comparison of alleles between NODAT and non-NODAT

Pearson Chi- square, odds ratio and confidence interval for comparison of alleles between general population and total patients

CI: 1.08–1.11). Many researchers obtained the same result [14, 32]. Multivariate ordinal logistic regression in Danish renal recipients showed that pre-transplantation insulin sensitivity index and age were the only predictive factors for NODAT [33]. In Korean kidney recipients, age at transplantation was found the only predictor for NODAT in multiple logistic regression analysis [34]. Whereas liver donors' age did not influence NODAT in 211 French liver-recipients in a trial [13]. In the present study, donors' data was not available.

We found no significant difference in NODAT frequency between males and females. Despite supporting some previous findings [11, 12], it was against some others where male sex was found a notable risk factor for NODAT [10]. A study stated a noteworthy point, implying that being male is only a risk factor for NODAT when it is accompanied by some cardiovascular risk factors too. Therefore, it is not clear yet if sex is an independent risk factor for NODAT or not [13].

The role of BMI is contradictory in various studies. Whilst it is reported as one of the strongest risk factors in some studies [2, 13], others failed to detect a significant relationship [10], as same as our study. However, some emphasize that waist to hip ratio may be more or as important as BMI [2]. A meta-analysis of seven studies (1046 patients) concluded that BMI seems to be a risk factor for NODAT. In these studies the mean±SD pre-transplantation BMI ranged from 22.6±2.0 to 29.2±5.1 kg/m^2 [10].

A Japanese study revealed no significant difference in mean pre-transplant BMI between those with and without NODAT. It was in line with our findings. In their research, 39 (24.2%) patients had a BMI >25 kg/m^2; only seven (4.3%) had BMI >30 [35]. In our study, 33 (27.6%) patients were overweight (25≤BMI≤29.9 kg/m^2) and only four (3.0%) had a BMI ≥30 kg/m^2. It might imply that the mean pre-transplant BMI in our study was not high enough to induce NODAT. Meanwhile, Vaughn, *et. al.*, did not discover any significant differences between the two groups, in spite of higher mean BMI than what we found [2]. These are in contradiction with the fact that BMI appears to affect development of type II diabetes in general population [36]. Furthermore, there was no significant relationship between NODAT and recipients' blood group (p=0.09), replicating the results of a previous study among liver recipients in an Iranian population [11]. An analysis on 82,104 women participating in E3N study, a French cohort initiating in 1990, however, showed that women with blood group O carry a lower risk of diabetes mellitus [37].

Table 6: Binary logistic regression analysis among factors supposed to be effective on NODAT in univariate analysis

Parameter	OR (95% CI)
Age	1.061 (1.081-1.106)
Prednisolone dose	1.301 (1.165-1.451)
MMF dose	0.522 (0.194-1.404)
M235T(1)[a]	7.326 (2.002-26.805)
T174M	
T174M(1)[b]	0.035 (0.001-0.813)
T174M(2)[c]	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 genotype with TT in diabetics than non-diabetics

T174M(2): Comparison of MT genotype with TT in diabetics than non-diabetics

In this survey, no association was found between NODAT and diseases that leading to transplantation (p=0.436). This was against many previous findings showing that hepatitis C is an important risk factor [13]. In fact, hepatitis C virus was expected to intensify NODAT risk, owing to its improvement of tumor necrosis factor secretion leading to insulin resistance, reducing the expression of insulin receptor substrates 1 and 2, and enhancing their degeneration.

No association was also found between the recipients' MELD score and development of NODAT (p=0.162). This is in line with many previous studies [2, 12].

A frequent adverse effect of prednisolone, a common corticosteroid, is hyperglycemia [38].

The mean prednisolone dose administered to those with NODAT was significantly higher than that in those without. In most studies, as same as ours, higher doses of prednisolone is associated with a higher risk of developing NODAT [9, 39]. Nonetheless, there are others who failed to find such relationship [40, 41]. Binary logistic regression analysis showed that an increase of 1 mg/day in prednisolone dose increases the likelihood of NODAT by 1.30 times compared with non-NODAT group (95% CI: 1.165–1.451, p<0.001). The risk of NODAT in Norwegian renal transplant recipients increased by 5% for each 0.01 mg/kg/day addition in prednisolone dosage. A significant relationship was found between 2-hour blood glucose and its dose in univariate and multivariate linear regression, and also between impaired glucose tolerance and prednisolone dose in multivariate model [42]. Besides, a significant linkage was observed between decreasing prednisolone dose to 5 mg/day and less serum glucose in renal transplant recipients in Norwegians [43].

It has been expressed that tacrolimus dose is an effective factor on NODAT in African-Americans. The mean±SD tacrolimus dose was 3.31±1.19 mg/day in non-NODAT and 3.61±1.85 mg/day in NODAT group, in our study (p=0.287). Although many studies found a significant association between tacrolimus dosage and development of NODAT [1, 9, 44, 45], many studies, including the current study, did not find this relationship [11, 41].

There is a possibility that race be a stronger risk factor than tacrolimus dose [7, 14]. Tacrolimus binds FK506-binding protein 12 and inhibits calcineurin, thereby, blocks insulin gene's transcription [34].

On the other hand, it seems that the serum level of tacrolimus is in fact more important than its dose, what was also shown in other studies [45, 46]. In this investigation, the mean blood tacrolimus level was 14.04 ± 4.23 ng/mL in NODAT and 8.21 ± 3.06 ng/mL in non-NODAT group (p<0.001). There are some studies showing no negative influence of serum tacrolimus trough level on NODAT [11, 40]. This parameter has not been entered regression model, since there were too many missing values in the data set.

In the population investigated, univariate analysis showed that MMF dose had a significant role in development of NODAT. This association, however, vanished after adjusting for confounding variables. In some research studies, it has been mentioned that using MMF may affect the development of NODAT [47]; other studies, however, failed to show any associations [6]. The frequency of hyperglycemia, as a complication of MMF use, ranges from 44% to 47% [48].

In evaluating AGT polymorphisms, this study found that MT+TT genotypes in M235T had a significant association with increased risk of NODAT. Although, binary logistic regression identified that T174M was a significant factor, it could not be reliable, as the confidence interval was too wide (CI 95%: 0.001– 0.813). This may be due to insufficient subjects with MM or TT genotypes in NODAT or non-NODAT groups.

We found that the frequency of M allele in M235T polymorphism in the control group was significantly higher than that in the transplanted arm. Research studies on M235T polymorphism in Nigerian and Turkish normotensive populations reported a T allele frequency of 96% [24], and 50% [23], respectively. This allele frequency reported for AGT T174M in Turkish normotensives was 85.6% [23]; it was 78.8% in our general population. In addition, a review of 44 studies conducted on whites, blacks, Asians, and mixed races, stated that normal population had an M allele frequency in M235T polymorphism of 51.5% and an M allele frequency in T174M 10.5% in 12 studies [49].

We found that M235T T allele increased the risk of NODAT twice; T174M wild allele increased the risk by 2.6-fold.

To the best of our knowledge, these two polymorphisms had not been investigated in post-liver transplantation diabetes yet. M235T was

found to be ineffective, but CT+TT of T174M were found to be markers in new-onset diabetes after renal transplantation in Korean population. This claim was the first to show a significant relationship between T174M and a rise in NODAT risk [3]. In addition, one study reported that Polish male renal recipients with TT genotype of M235T have a higher concentration of insulin, but not lower risk of NODAT [50]. Another study found that the wild genotype (TT) of M235T polymorphism might lead to higher incidence of NODAT in Turkish renal recipients compared to those with mutant genotypes [51]. The difference observed between the results of this research and previous studies may be related to the subjects' race or the sample size investigated. This study's subjects were close to southwestern Asians and Europeans, especially Greeks and Italians, in terms of HLA typing [52].

There were some limitations in this research, including limited data or unavailability of some patients' documents and donor data. However, the study power obtained confirmed that the sample size was reasonably enough.

In conclusion, we found that age, prednisolone dose, and MT+TT genotypes of M235T are predictors of NODAT in Iranian liver transplant recipients.

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