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Research Article

Assessment of the Effect of HMGCR Variant Alleles on Response to Atorvastatin Treatment in Type 2 Diabetic Egyptian Patients

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Abstract. Use of 3 hydroxyl-3-methylglutaryl-3-coenzyme A reductase (HMGCR) inhibitors, or statins, reduces the progress and the complications of diabetes mellitus by modifying the lipid profile. The aim of this study was to assess the association between variation in statin response and the single-nucleotide polymorphism (SNP) rs12916 C/T in the gene encoding HMGCR, a rate limiting enzyme in cholesterol synthesis and the target enzymatic reaction of statins. A total of 96 Egyptian patients with type 2 diabetic dyslipidemia were treated with atorvastatin 20 mg/day for 3 months. Total cholesterol, triglyceride, low density lipoprotein–cholesterol (LDL-C), high density lipoprotein–cholesterol (HDL-C) plasma concentrations were measured at baseline and at the end of the treatment period together with genetic screening for SNP rs 12916 C/T. It was found that individuals with the CC genotype showed a mean reduction in LDL-C level of about 31.57 ± 87.52 mg/dl (p=0.24), while the reduction in the CT and TT genotypes was 28.50 ± 72.74 mg/dl (P=0.04) and 26.15 ± 101.45 mg/dl (p=0.06), respectively. It is concluded that there is no significant association between this SNP rs12916 C/T and response to atorvastatin therapy in type 2 dyslipidemic diabetic Egyptian patients.

Keywords: HMGCR, polymorphism, lipids, atorvastatin, diabetes mellitus.

1. Introduction

Statin treatment is recommended for lowering low-density lipoprotein cholesterol (LDL-C) levels in patients with type 2 diabetes that have cardiovascular disease or type 2 diabetic patients above the age of 40 years with cardiovascular risk [1]. The advantageous effects of statins are that they are generally well tolerated and decrease the synthesis of endogenous cholesterol by reversibly inhibiting 3-hydroxy-3-methyl glutaryl Co enzyme A reductase (HMGCR), mainly in hepatic cells [2]. However, the magnitude of LDL-C reduction from statin treatment demonstrates high interindividual variability [3]. Phenotypic and genetic factors can affect the response to statin. Adherence to the prescribed regimen, smoking, age, sex, body weight, diet, physical activity, and baseline plasma LDL-C concentrations are non-genetic contributors to statin response [4]. Gene polymorphism in HMGCR is one of the main genetic factors that affect the outcomes of statin therapy [5].

Atorvastatin is a potent HMGCR inhibitor and lipid regulating agent used for treating patients with hyperlipidemia. It reduces LDL-C, apolipoprotein B, and triglyceride concentrations, and increases levels of high- density lipoprotein cholesterol (HDL-C). It is also utilized as a prophylactic drug for cardiovascular events in patients with multiple risk factors including diabetes mellitus [5, 6].

This clinical trial examined the relationship between the polymorphism rs 12916 C/T in HMGCR gene and the LDL-C lowering effect of atorvastatin therapy in Egyptian patients with type 2 diabetic dyslipidemia.

2. Materials and Methods

2.1. Patients. The study was conducted on 100 patients who were recruited from the diabetes clinics of the Alexandria University Hospital after giving written consent. Four patients subsequently discontinued the trial as they did not attend the follow-up visit. The participant patients suffered from type 2 diabetes mellitus and dyslipidemia, their age ranged between 30–70 years, and they were not receiving any lipid lowering agent at baseline. Patients with history of nephrotic syndrome, chronic renal disease, treatment induced dyslipidemia, and liver diseases were excluded from the study. The clinical trial was approved by the Institutional Review Board of the medical school in Alexandria university. Baseline health, demographic data, anthropometric measurements including body weight and body height, physical examination, and laboratory investigations were obtained on enrollment. The lipid profile (total cholesterol, triglycerides, HDL-C, LDL-C) was also obtained. Those diabetic patients who were dyslipidemic with normal liver and renal functions, then received 20 mg atorvastatin (Ator tablet 20 mg, Epico, Egypt) daily for three months. After this treatment period, the patients were assessed for polymorphism rs 12916 C/T in the HMGCR gene and a second lipid profile (total cholesterol, triglycerides, HDL-C, LDL-C) was obtained.

2.2. Laboratory investigations. Blood samples were collected from 12 hour fasting patients who had been fasting for at least 12 hours. Serum cholesterol (TC), triglycerides (TG), HDL-C, and LDL-C levels were measured using standard enzymatic kits (N.S. BIO-TEC/London, UK). LDL-C concentrations were calculated as total cholesterol minus cholesterol in the supernatant as measured by the precipitation method [7].

Molecular screening for polymorphism rs 12916 C/T in HMGCR gene was done by DNA extraction; using the PURE LINK ®genomic DNA extraction kit (Invitrogen) (Germany) and Polymerase Chain Reaction; utilizing Taq Man® Universal Master Mix II (Applied Biosystems-USA) [8].

2.3. Statistical analysis. Data were analyzed using the IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation, and median. Significance of the obtained results was judged at the 5% level [9, 10]. Patients were divided into statin responders and non-responders on the basis of the significance reduction of LDL-C and TC levels after atrovastatin treatment (20 mg/day for 3 months) The chi-square test was used to compare categorical variables between different groups. Fisher's exact or Monte Carlo correction for chi-square were applied when more than 20% of the cells had an expected value less than 5. For normally distributed variables, Student's t-test was utilized to compare results from the two different study groups and paired t-tests were applied to compare results between two periods in the same studied group. For non-normally distributed variables, the Mann Whitney test was used to compare data from the two studied groups and Wilcoxon signed ranks test was used to compare between two periods in a single study group. The Spearman coefficient was used to show the association between change in lipid profile (TC, HDL-C, LDL-C, TG) and sex, age, and body mass index (BMI) of the participant.

3. Results

Ninety six patients completed the study after they had received atorvastatin therapy 20 mg/day for three months and their response to treatment was evaluated by measuring lipid parameters. The compliance of the patients was evaluated by coming with the remaining pills or the empty boxes when the patients visited the clinic for follow-up. The patients were divided into 2 groups according to their level of reduction in LDL-C and TC after statin treatment using paired t-test. Thirty seven and half percentage of patients (n=96) were observed to be responders (36 patients) and 62.5% were non-responders (n=60). The baseline of our patients had very high of LDL-C concentration (>190 mg/dL) and high level of total cholesterol (>240 mg/dL)The responders patients to moderate intensity therapy of atorvastatin therapy

(20 mg/day) who achieved LDL-C concentration ≤ 190 mg/dL.

Baseline characteristics of the 96 patients are presented in Tables 1 and 2. As shown in Table 3, analysis of variant alleles of HMGCR gene data revealed that homozygous CC group contained 14 patients representing 14.6% of the total population divided as follows 7 patients in the non-responder group constituting 11.7% and 7 patients in the responder group representing 19.4%, while homozygous TT included 54 patients representing 56.3% of the whole sample size, 35 of them belonging to non-responder group constituting 58.3% and 19 patients in the responder group representing 52.8% while heterozygous CT were 28 patients representing 29.2%of the total participants divided as follows 18 in the non-responder group, representing 30%, and 10 in the responder group constituting 27.8% Allele frequency of C was 56 which represents 29.2.% of the sample size while allele frequency of T was 136 which represents 70.8%. of the study population. Based on the Chi square test and Monte Carlo correction test there was no significant difference in the genotype of the responder and non-responder groups. Regarding the relation between the genotype of SNP rs12916 C/T in the HMGCR gene and the reduction LDL-C level shown in Table 4, there is no significant reduction of LDL-C before and after treatment with moderate intensity therapy of atorvastatin that can be correlated with The SNP rs12916 C/T by using ANOVA test and Chi square for Kruskal Wallis test ($P \geq 0.05$).

In Table 4, individuals with the CC genotype showed a mean difference reduction in LDL-C level of about 31.57 ± 87.52 mg/dL, while the reduction in CT and TT genotype was 28.50 ± 72.74 mg/dL and 26.15 ± 101.45 mg respectively. However, it was found that there is significant correlation between before and after treatment in CT genotype only of this polymorphism by using paired t-test ($P \leq 0.05$). But this only correlation has no significant importance statistically and clinically.

Concerning the changes in lipid profile, there is a highly significant difference change in the levels of TC, TG, HDL-C and LDL-C as shown in Figure 1 before and after the treatment using paired t-test ($p \leq 0.001$). However, in Table 5, there is no significant correlation between age and body mass index (BMI) of the participants and the change in lipid profile (TC, TG, HDL-C, LDL-C). Non-smoking patients showed better improvement with atorvastatin treatment than smokers mainly in LDL-C level and in TC level (Table 6).

Table 1: Baseline clinical characteristics of the 96 study patients.

	Mean	**± SD**
Age (years)		
Range (30–70)	54.41	6.99
Body Weight (kg)	89.81	12.12
Height (cm)	166.38	7.36
Body mass index (kg/m^2)	32.46	3.95
Waist (cm)	100.56	9.40
LDL-C (mg/dl)		
Baseline	206.97	90.48
After 3months	179.34	55.47
Change	27.62	91.09
TC (mg/dl)		
Baseline	278.0	92.06
After 3months	249.99	58.22
Change	28.01	90.18
HDL–C (mg/dl)		
Baseline	32.42	13.48
After 3months	45.82	15.59
Change	13.41	19.64
TG (mg/dl)		
Baseline	191.90	106.97
After 3months	140.50	70.04
Change	-51.40	99.79
Liver function		
SGOT (U/L)	29.30	11.85
SGPT (U/L)	40.46	16.90
Renal function		
Blood Urea (mg/dl)	35.42	13.63
Serum Creatinine (mg/dl)	0.83	0.2
FBG (fasting blood sugar mg/dl)	182.90	87.57
HB (g/dl)	12.31	1.38

Table 2: Distribution frequency of key clinical characteristics in the 96 study patients.

	No.	**%**
Sex		
Male	34	35.4
Female	62	64.6
Hypertension	58	60.4%
Diabetes	96	100%
Smoking	16	16.66%

Table 3: Comparison of statin response according to genotyping and allele frequency.

	Total (n = 96)		Response				χ^2	P
			Non responder (n = 60)		Responder (n = 36)			
	No.	%	No.	%	No.	%		
Genotyping								
CC	14	14.6	7	11.7	7	19.4	1.098	^{MC}p= 0.90
CT	28	29.2	18	30.0	10	27.8		
TT	54	56.3	35	58.3	19	52.8		
Allele								
C	56	29.2	34	27.1	22	30.6	0.260	0.61
T	136	70.8	86	72.9	50	69.4		

χ^2: Chi square test

MC: Monte Carlo

C: Normal allele.

T: Abnormal allele: where SNP is present

CC: Homozygous normal.

CT: Heterozygous abnormal.

TT: Homozygous abnormal.

Table 4: Relation between genotype and LDL –C response.

LDL-C (mg/dl)	Genotype			Test of sig.	p
	CC (n= 13)	CT (n=28)	TT (n= 55)		
Before					
Mean ± SD.	221.7 ± 89.92	202.9 ± 83.45	206.9 ± 95.58	F= 0.405	0.67
After.					
Mean ± SD.	191.08 ± 57.70	174.43 ± 58.39	155.04 ± 7.13	F= 0.193	0.83
^{t}**p**	0.25	0.05*	0.06		
Mean difference					
Min. – Max.	-109.0 – 223.0	-107.0 – 159.0	-214.0 – 303.0		
Mean ± SD.	31.57 ± 87.52	28.50 ± 72.74	26.15 ± 101.45	$^{KW}\chi^2$= 0.086	0.96
Median	2.0	13.0	35.50		

F: F value for ANOVA test

$^{KW}\chi^2$: Chi square for Kruskal Wallis test

^{t}p: p value for Paired t-test for comparing between before and after in each other group

*: Statistically significant at p ≤ 0.05.

Table 5: Correlation of age and body mass index (BMI) with change in lipid profile.

Change	Age		BMI	
	r_s	P	r_s	p
LDL-C (mg/dl)	-0.067	0.52	0.067	0.72
HDL-C (mg/dl)	0.039	0.71	-0.078	0.68
TG (mg/dl)	-0.004	0.97	-0.245	0.18
TC (mg/dl)	-0.055	0.60	0.080	0.66

rs: Spearman coefficient

There is no significant association between age of the participant, Body mass index (BMI) and change in lipid profile (TC,HDL-C,LDL-C,TG)

Table 6: Correlation of smoking with change in lipid profile.

Change	Nonsmoker (n = 81)	Smoker (n = 15)	Z	P
LDL-C (mg/dl)				
Min. – Max.	-214.0 - 303.0	-6.0 - 213.0		
Mean ± SD.	14.25 ± 88.20	99.87 ± 72.31	3.572*	<0.001*
Median	12.0	95.0		
HDL-C (mg/dl)				
Min. – Max.	-43.0 - 60.0	-19.0- 46.0		
Mean ± SD.	14.80 ± 19.94	5.87 ± 16.52	1.948	0.05
Median	15.0	6.0		
TG (mg/dl)				
Min. – Max.	-515.0 - 148.0	-249.0- 123.0		
Mean ± SD.	-51.70 ± 102.81	-49.73 ± 84.73	0.116	0.91
Median	-42.0	-42.0		
TC (mg/dl)				
Min. – Max.	-170.0- 341.0	-24.0 - 202.0		
Mean ± SD.	16.25 ± 88.29	91.53 ± 74.29	3.159*	0.002*
Median	13.0	106.0		

Z: Z for Mann Whitney test

*: Statistically significant at $p \leq 0.05$

There is significant effect of the smoking on change in lipid parameters after being treated with atorvastatin therapy mainly on LDL-C level and on TC level.

Non smoker show better improvement than smoker.

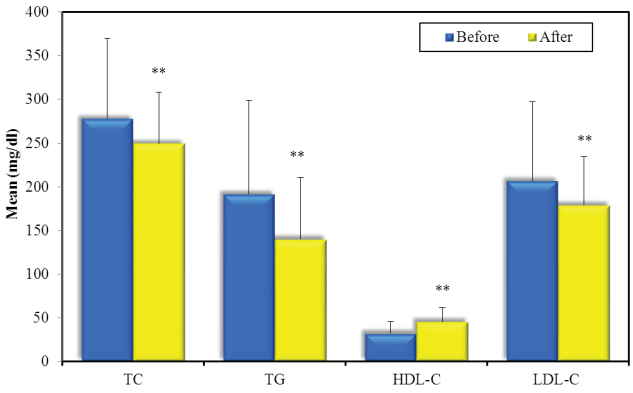

Figure 1: Comparison between the two studied groups (before and after treatment) according to the level of TC, TG, HDL-C and LDL-C (mg/dl). (^{t}p: p value for Paired t-test for comparing between before and after in each other group; **: Statistically high significant at $p \leq 0.001$.)

4. Discussion

Statins are considered one of the most effective drugs for reducing LDL-C and TC [11, 12]. Phenotypic and genetic factors play an important role in the variation of LDL-C levels after statin therapy. Phenotypic predictors include age, sex, obesity, smoking status, physical activity and ancestry. However, single nucleotide polymorphisms and haplotypes are genetic factors also associated with statin response [13].

Older patients above 60 years, have a greater response to statin therapy with a greater reduction in LDL-C level than younger adults [14, 15]. In this study the age of the participants ranged from 30 to 70 years. However, age did not appear to be significantly associated with the change in TC,TG, LDL-C and HDL-C levels after atorvastatin treatment. Female sex is associated with higher levels of HDL-C and slightly lower LDL-C concentrations than are generally found in males [14]. In this clinical trial, there was no correlation between the sex and change in lipid parameters, possibly a consequence of the small sample size. Other phenotypic factors that we examined are obesity and smoking. The participants in our study were obese with a mean BMI of 32.46 kg/m^2. Nevertheless we did not find any correlation between obesity and response to atorvastatin 20 mg/day as evaluated by change in the lipid profile. On the other hand, smoking did affect lipid levels, especially TC and LDL-C. It was found that non-smokers exhibited better improvement than did smokers, this may be due to induction of cytochrome P450 which is responsible for statin metabolism [16].

Concerning genetic factors, this study was designed to examine the effect of SNP rs12916 C/T in HMGCR gene on statin response in type 2 diabetic dyslipidemic Egyptian patients who were treated with atorvastatin in a dose of 20 mg/day for three months but no association was found. This contrasts with the results found by Chien et al. who studied primary hypercholesterolemic Chinese patients who received various statins for 9 months to elucidate the effect of polymorphism in ten candidate genes involved in pharmacokinetic and pharmacodynamic statin pathways [17]. They reported that individuals with CC genotype (SNP rs 12916 C/T in HMGCR gene) showed a reduction of 56.9 mg/dl for LDL-C, with the reduction increasing to 60.1 and 62.5 mg/dl among individuals carrying CT and TT, respectively [17]. Poduri et al. conducted a clinical trial in 265 Indian patients with newly diagnosed coronary artery disease who received atorvastatin (20 mg/day) and were followed up to 6 weeks [18]. Multiple SNPs were examined, three of them at the level of the HMGCR gene, namely rs 12916 C/T, SNP 29 G/T, and rs 5908 A/G. It was found that the rs 12916 C/T SNP variant was significantly associated with LDL-C reduction where as the wild type of this SNP exhibited significantly lower reduction of LDL-C levels after atorvastatin therapy. Chung et al. investigated in healthy Korean participants the relationship between polymorphism rs3846662 in the HMGCR gene and the LDL-C–lowering effects of atorvastatin (20 mg/day) [19]. They discovered that The HMGCR rs3846662 GG genotype was significantly associated with higher basal LDL-C levels and a possibly greater response to atorvastatin. Moreover, it was reported that in Chilean hypercholesterolemic individuals, HMGCR rs17671591 SNP influences the lipid lowering therapy with atorvastatin 10 mg/day for 4 weeks [20]. Regarding racial differences, other investigators found that blacks are highly affected by HMGCR gene polymorphisms that are associated with decreased LDL-C level in response to simvastatin therapy 40 mg daily for 6 weeks than white people [21].

In conclusion, the discrepancy between the results of previous studies and what we have found may be attributed to the homogenous small sample size and the assessment of the single rs 12916 C/T SNP in the HMGCR gene, which may not affect the response to atorvastatin therapy if present alone. Further studies in various population groups of different race and ethnicity are necessary to more fully characterize the effects of different genotypes on the clinical efficacy of statin therapy.

Competing Interests

The authors declare no competing interests.

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