Effect of GATM Gene Polymorphism in the Incidence of Myopathy Among Iraqi Patients Treated with Atorvastatin in Kerbala Province

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Abstract

Background: Statins are a class of drugs that block the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase, preventing de novo cholesterol synthesis. They are the most commonly prescribed lipid-lowering drugs in the world for the primary and secondary prevention of cardiovascular disease. Statin-associated muscular symptoms are the most common side effects of statin therapy, forcing patients to discontinue their medication. The Glycine amidinotransferase (GATM) gene codes for the mitochondrial enzyme L-arginine: glycine-amidinotransferase, a rate-limiting enzyme in creatine biosynthesis. Creatine is then necessary for normal muscle function. Statins have been shown to reduce GATM expression and thus creatine content in muscles, which may contribute to statin myopathy Aim of study: To investigate the relation between GATM gene polymorphism rs9806699 G > A, C, T and statin-related myopathy (SRM) in patients taking atorvastatin 40 mg. Patients and methods: One hundred fifty Iraqi male and female patients aged 28 to 65 years who were taking atorvastatin 40 mg once daily were chosen to participate in this cross-sectional study. Thyroid stimulating hormone and creatinine kinase were measured. The allele specific polymerase chain reaction technique was used to detect the rs9806699 G > A, C, T single nucleotide polymorphism (SNP). Results: The genotypes distribution of rs9806699 G > A, C, T was 20 (13.3%), 57 (38.0%) and 73 (48.7%) for homozygous wild (GG), heterozygous (GA), and homozygous mutant (AA) respectively, with no allele frequency for C and T. Despite a significant increase in mean creatine kinase in homozygous mutant (AA) patients compared to wild-type (GG) or heterozygous (GA) patients, there was no significant association between statin-related myopathy and GATM gene rs9806699 polymorphism. Conclusion: Although the rs9806699 SNP of the GATM gene is not associated with statin-related myopathy, it cannot be ruled out as one of the factors that contribute to myopathy since we observed that the A allele was found in more SRM patients than the G allele, with a significant increase in the mean creatine kinase level.

Keywords: Myopathy, Atorvastatin, Glycine, Amidinotransferase (GATM) gene, rs9806699, Creatine.

1. Introduction

Myopathies are skeletal muscle disorder characterized by a primary functional or structural dysfunction. The most prevalent signs and symptom include weakness, cramps, stiffness, and spasms(1). Myopathies can be acquired or hereditary, and they can develop at any age (2). Many chemicals, including routinely prescribed pharmaceuticals, might cause muscular damage (3,4). In recent years, cholesterol-lowering drugs, particularly statins, have been the most often prescribed treatments that have been linked to myopathy (5,6). Statins are a class of drugs that block the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase, which prevents de novo cholesterol synthesis, and they are the most widely prescribed lipid-lowering drugs in the world for the primary and secondary prevention of cardiovascular diseases (CVDs) (7,8), which are presently the leading cause of mortality worldwide (9). Despite the beneficial effects of statin treatment on cardiovascular health, patient compliance is frequently low (10). The most prevalent side effects of statin therapy are myotoxicity. All statins can cause myotoxicity, which is dose-dependent and can force a patients to stop taking their medication (11). The American College of Cardiology/American Heart Association/National Heart, Lung, and Blood Institute (ACC/AHA/NHLBI) developed terminology used to categorize statin-related muscle adverse effects including myalgia, asymptomatic increases in creatine kinase (CK), myositis, and rhabdomyolysis (2), as summarized in table 1. The lack of agreement in the definition of SRM makes exact assessment of their real incidence difficult (12). Furthermore, the risk of statin myopathy in clinical trials (1.5-5%) is much lower than in observational studies (10-33%) (13).

Table 1: Different terms used by the ACC/AHA/NHLBI to describe skeletal muscles disorders (2).

Condition	Definition
Myopathy	A collective term for any skeletal muscle- related side effects.
Asymptomatic	Elevation of creatine kinase (CK) without muscular symptoms.
Myalgia	Weakness or pain in muscle without creatine kinase (CK) elevation.
Myositis	Weakness or pain in muscle associated with creatine kinase elevation (CK).
Rhabdomyolysis	Muscle symptoms associated with significant creatine kinase (CK) elevation (>10 times of upper limit of normal) and myoglobinuria as a result of muscular destruction.

CK: creatine kinase.

doi.org/10.31838/hiv23.02.121 Reaceived: 16.10.22, Revised: 17.11.22, Accepted: 19.12.22.

Glycine amidinotransferase gene (GATM) located on chromosome 15q15.3, It encodes the mitochondrial enzyme L-arginine: glycine-amidinotransferase (AGAT or GATM), which is a rate-limiting enzyme involved in the biosynthesis of creatine by converting arginine and glycine to ornithine and guanidinoacetate (GAA) (14). A mutation in the GATM gene, which has a length of 41,203 base pairs (bp), results in hereditary creatine deficiency syndromes, which are also characterized by severe mental retardation, speech delay, epilepsy, autism, and hypotonia (15). Creatine is needed for normal muscular function. Creatine kinase within the muscle phosphorylates creatine to generate a pool of phosphocreatine (PCr). The N-phosphocreatine pool stores energy for ATP replenishment during periods of high energy demand (16). Thus, phosphocreatine can transfer its phosphate group to adenosine diphosphate (ADP) to resynthesize ATP, a step mediated by the creatine-kinase enzyme (17). Mangravite et al. reported the first association between GATM rs9806699 G > A polymorphism and SRM in a case control study with 72 SRM cases in 2013. The A allele was linked to a decrease in GATM expression, which resulted in a decrease in creatine synthesis. Reduced creatine availability may affect energy metabolism in skeletal muscle cells, contributing to the pathogenesis of myopathy induced by statin (18). However, the following studies on the effect of rs9806699 G > A on SRM produced contradictory results (19–22). As a result, the role of GATM polymorphism in SRM remains a hotly debated topic to this day. The aim of this study is to investigate the relation between GATM gene polymorphism rs9806699 G > A, C, T and statin-related myopathy in Iraqi patients taking atorvastatin 40 mg.

2. Materials and Method

Study participant and overall design

Cross-sectional observational non-interventional study was conducted between December 2021 and April 2022 at Kerbala's Imam Al-Hussein Medical City's Cardiology Center, Endocrinology and Diabetes Center, as well as Al-Zahraa Center. The Scientific and Ethical Committee of Pharmacy College/Kerbala University approved the study's protocol, and each subject signed an informed consent form after being informed of the study's nature and objectives. The study included 150 male and female patients ranging in age from 28 to 65 years old and took 40 mg of atorvastatin orally each day as monotherapy for hyperlipidemia for at least one month. The classification system described by ACC/AHA/NHLBI was used in this study to define statin-related myopathy (SRM) (2).

Patients with severe renal, hepatic, or cardiac dysfunction, untreated hypothyroidism or hyperthyroidism, advanced age (>65), recent surgery or trauma, taking atorvastatin-interacting medications (such as fibrates, nicotinic acid, niacin), vigorous exercise, or taking dietary supplements containing creatine monohydrate are excluded from this study.

Blood samples were collected from patients and divided into two parts: the first (2 mL) was placed in the ethylene diamine tetra acetate (EDTA) tube for DNA extraction, and the second (3 mL) was placed in a gel tube for subsequent analysis of serum thyroid stimulating hormone (TSH), serum creatinine, and creatinine kinase (C.K) level. Before being transported to the laboratory for analysis, the samples were stored in ice-filled containers to maintain the proper temperature.

Genetic analysis

Genomic DNA was extracted from whole blood using a genomic DNA extraction kit according to the manufacturer's instructions (geneaid, Taiwan). Each extraction yielded a total volume of 100 µl of genomic DNA. Nanodrop was used to determine the purity and concentration of extracted DNA samples.

Allele specific Polymerase Chain Reaction (AS-PCR) was used to amplify the GATM gene rs9806699 G > A, C, T using specific primers purchased from Alpha DNA company/Canada as lyophilized products in various picomole concentrations. The sequences of the primers were as follows: M-ALLELE G: AATGTCACCATGCCCCAGAGC, M-ALLELE A: AATGTCACCATGCCCCAGAGT, M-ALLELE T: AATGTCACCATGCCCCAGAGA, M-ALLELE C: AATGTCACCATGCCCCAGAGG and M-Reverse primer: TGCGCCTTCCTGGTGTTCAT.

The PCR reaction mixture contained 3 µL (100 ng/ µL) of genomic DNA, 1 µL (2.5 pmol/ µl) for each Forword and reverse primer, 15 µL of nuclease free water and 5 µL of bioneer's PCR premix. The following program was used in the thermocycler: initial denaturation for 5 minutes at 95°C, 30 cycles of amplification (denaturation for 20 seconds at 95 °C, annealing for 10 seconds at 61.5 °C, extension for 15 seconds at 72 °C, and final extension for 5 minutes at 72 °C), and final extension for 5 minutes at 72 °C. The amplification was verified by electrophoresis using a 1.5% (w/v) agarose gel stained with ethidium bromide (0.5 mg/ml).

Statistical analysis

The data of the present study was entered and analyzed through the Statistical Package for the Social Sciences (SPSS version 24). The data were presented as frequencies and percentages or mean and standard deviation in appropriate tables and graphs. Chi square test, Fisher's exact test, ANOVA test, T test and post hoc analysis were used where is appropriate to find out the possible association between the related variables of the current study. Statistical association considered significant when p value equal or less than 0.05.

3. Results

The age of the included patients (N=150) ranged from 28 to 65 years with a mean of 50.9±9.2 years. Female to male ratio was 1.2:1. More than two thirds

of the patients of the present study reported the use of the drug for at least one year. More than one half of the patients (56%) had no Statin Related myopathy as shown in table 2.

Table 2: Socio-demographic and some related characteristics of the included participants.

Characteristics		Total=150 No. (%)
Age (in years)	mean ±SD	50.9±9.2
	Range	28- 65
Age groups (years)	< 40	22 (14.7)
	40- 50	49 (32.7)
	51-60	59 (39.3)
	>60	20 (13.3)
Gender	Female	81 (54)
	Male	69 (46)
BMI	Normal weight	42 (28)
	Overweight	70 (46.7)
	Obese	38 (25.3)
Duration of treatment (months)	1-11	44 (29.3)
	12-23	41 (27.3)
	24-36	40 (26.7)
	>36	25 (16.7)
Smoking	Yes	41 (27.3)
	No	109 (72.7)
Diabetics	Yes	50 (33.3)
	No	100 (66.6)
SRM	Asymptomatic	19 (12.7)
	Myalgia	29 (19.3)
	Myalgia with mild C.K elevation	18 (12)
	Normal	84 (56)

BMI: Body mass index, N: Numbers of the Study participant, SD: Standard deviation, SRM: statin related myopathy, C.K creatine kinase.

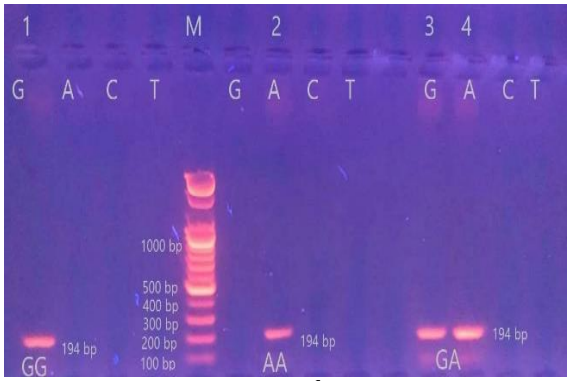

Figure 1: Genotyping of rs9806699 genetic polymorphism showed: lane M represent DNA ladder 100 – 1500 bp, lane 1 represent GG genotype (wild), lane 2 represent AA genotype (mutant) and 3 and 4 lanes represent GA genotype (heterozygous).

AS-PCR was used to detect the, rs9806699 G > A, C, T SNP on GATM gene and produced a clear band with a molecular size of 194 bps (Figure 1). The size of the amplicon was estimated by comparing it to a 100-1000 bp DNA ladder.

The patients in this study were divided into three genotypes for the GATM gene rs9806699 (G>A, C, T) genetic polymorphism: homozygous for the G allele (GG) wild type, heterozygous (GA), and homozygous for the A allele (AA) mutant type. There were 20 GG genotypes (13.3%), 57 GA genotypes (38%), and 73 AA genotypes (48.7%) among 150 patients, with no allele frequency for C and T alleles in the Iraqi population table 3.

Table 3: Allele frequencies of GATM rs9806699 (G>A, C, T) gene polymorphism.

SNP	Genotypes	Frequency	percentage
rs9806699	GG	20	13.3%
	GA	57	38.0%
	AA	73	48.7%
	GC	0	0.0%
	CC	0	0.0%
	GT	0	0.0%
	TT	0	0.0%
Total		150	100%

The analysis of data showed that association of socio-demographic and some other characteristics of the included participants in relation to genotype were statistically not significant (p>0.05). There was no significant difference in the levels of TSH and serum creatinine, while there was statistically significant difference of mean Creatine kinase in relation to genotype; post hoc analysis concluded that AA allele responsible for this difference (table 4).

Table 4: Association of socio-demographic and mean biochemical parameters with genotype.

Variables	Categories	Genotype: No. (%)			P value
		GG (n=20)	GA (n=57)	AA (n=73)	
Age (years)	Mean ±SD	51.2±9.45	49.81±9.04	50.82±9.28	0.769
Gender	Female	14(17.3)	26(32.1)	41(50.6)	0.149
	Male	6(8.7)	31(44.9)	32(46.4)	
Smoking	No	15(13.8)	44(40.4)	50(45.9)	0.527
	Yes	5(12.2)	13(31.7)	23(56.1)	
SRM	Asymptomatic	0	6(31.6)	13(68.4)	0.236
	Myalgia	5(17.2)	10(34.5)	14(48.3)	
	Myalgia with mild C.K elevation	1(5.6)	6(33.3)	11(61.1)	
	Normal	14(16.6)	35(41.7)	35(41.7)	
TSH	Mean ±SD	1.71±0.88	1.95±1.56	4.16±16.07	0.468
CK	Mean ±SD	112.70±74.34	134.81±66.80	163.34±89.89	0.02*
SCr	Mean ±SD	0.89±0.89	0.80±0.30	0.87±0.45	0.657

The data is represented as mean± standard deviation, N: Numbers of the study participant, SRM: statin related myopathy, TSH: thyroid stimulating hormone, CK: creatine kinase, SCr: serum creatinine.

4. Discussion

Mangravite et al. firstly revealed a possible genetic marker for a lower risk of SRM. They found an association between expression quantitative trait loci (eQTLs) for the GATM (rs9806699 G > A) and simvastatin exposure using gene expression profiling of lymphoblastoid cell lines (LCLs). The rate-limiting enzyme in creatine production is glycine amidinotransferase. Creatine is primarily synthesized in the liver and kidneys and is then transferred to skeletal muscle, where it serves as a vital source of cellular energy. Simvastatin-exposed LCLs with the A allele showed a higher decline in GATM RNA expression than non-exposed control LCLs. As a result, decreased GATM expression should result in decreased creatine biosynthesis and creatine phosphate storage. They hypothesize that decreased creatine phosphate storage alters skeletal muscle cellular energy pathways, making them less susceptible to statin myopathy, and that the A allele is significantly associated with a lower risk of SRM (18). On the contrary, later research did not corroborate this conclusion. According to Carr et al., there was no significant different in the minor allele frequency between controls (n = 587) and myopathy patients (n = 150) (19). Moreover, utilizing a case-control study of SRM in 715 Caucasians, Luzum et al. were unable to replicate the protective role of GATM described by Mangravite et al (22). Other study was conducted in Japanese populations had similarly insignificant results (23). Our study support earlier studies by demonstrating that the GATM gene polymorphism (rs9806699) has no statistically significant impact on SRM (p=0.08). Despite the fact that our results did not meet the criteria for statistical significance most likely due to the multiple pathways that may affect atorvastatin pharmacokinetics and pharmacodynamics, we discovered that more patients with SRM had the A allele than the G allele. A statistically significant increase in the mean creatine kinase was also seen in homozygous mutant (AA) patients compared to wild-type (GG) or heterozygous (GA) patients, as shown in table 4 which summarizes the data. As a result, patients with the A allele may be at higher risk of SRM.

Statins, as previously stated, reduce GATM expression and hence creatine content in muscles (18). Several studies found that GATM deficit was linked to a myopathy in two patients, which improved with oral creatine therapy, suggesting that decreasing intramuscular creatine does not prevent, but rather caused, myopathy (24). Furthermore, in ten patients with statin myalgia, over the counter creatine supplementation appeared to minimize statin-associated muscular symptoms (25). Since creatine serves as the ultimate acceptor of the phosphate group of ATP at the ending of mitochondrial oxidative phosphorylation, a statin-induced drop in the intracellular concentration of creatine may result in mitochondrial dysfunction. Reduced ATP production by cells would come next. As a result, cells treated with statins produce less ATP. In vitro, statin-induced apoptosis is halted by creatine supplementation through preventing the opening of the mitochondrial permeability transition pore (26).

Creatine's role in sustaining appropriate muscular function is also supported by the observation that muscles in mice deficient the enzyme GATM had atrophy and lower strength. These animals had essentially little creatine in their muscles and many metabolic anomalies for example, their inorganic phosphate/-ATP ratio was fourfold higher, indicating reduced phosphate consumption in ATP production. These mice's muscles also exhibited morphological changes, including lipid droplets and unusual crystal formations in the mitochondria, along with a 70% reduction in muscle volume. Functionally, mice had a more than 70% reduction in muscle strength and were hypotonic. When creatine was added to the diet, the aforementioned abnormalities nearly returned to normal (27). In the ending, additional clinical trials with a large number of patients should be conducted in the goal of providing more clear information on the role of GATM gene polymorphism and statin myopathy risk, as well as the benefit of creatine supplementation in statin myopathy.

5. Conclusion

Glycine amidinotransferase gene was detected with different genotypes and variable frequencies in Iraqi patients that taking atorvastatin. The homozygous mutant type (AA) of GATM gene polymorphism rs9806699 G > A, C, T is more predominant than GG and GA genotype, with no allele frequency for C and T. Although the rs9806699 SNP of the GATM gene is not associated with statin-related myopathy, it cannot be ruled out as one of the factors that contribute to myopathy since we observed that the A allele was found in more SRM patients than the G allele, with a significant increase in the mean creatine kinase level.

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