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A Prospective Study to Estimate the Prevalence of SLCO1B1*5 and its Implications in Statin Induced Myopathy.

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ABSTRACT

Atorvastatin is the commonly used drug for hyperlipidemia. Myalgia is one of the common adverse effects of statins which decreases compliance. A single nucleotide polymorphism (SNP) in the gene coding Soluble Carrier Organic Anion Transporter-SLCO1B1*5 increases the risk for myalgia. Due to muscle injury Creatine Phospho Kinase (MM) levels are also increased. To determine the prevalence of SLCO1B1*5 allele and its association with myopathy due to atorvastatin. After getting informed consent, 192 patients on atorvastatin were included in the study. They were divided into 2 groups: patients on a lower dose (10mg/day) and on a higher dose (80mg/day). The basic demographic details and medical history were recorded. Their blood (5ml) was collected and analyzed for SLCO1B1*5 and levels of CPK(MM). The variant allele C was present in 35% of study group. The odds ratio for myalgia in CT heterozygotes in lower dose is 1.5873 and 12.80 in higher dose as compared to TT homozygotes; the odds ratio for CC homozygotes in lower dose is 18.75 and 43 in higher dose as compared to TT homozygotes. The presence of C allele increases the risk of myalgia and CPK MM level increases in CT and CC variants.

Keywords: Atorvastatin, myalgia, SLCO1B1*5, CPK (MM).

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INTRODUCTION

Atorvastatin is one of the hypolipidaemic drugs which belong to the class commonly known as statins. Statins or HMG COA reductase inhibitors are the first line hypolipidaemic drugs. Myalgia is one of the common adverse effects of statins. Due to myalgia some patients stop taking the drug.

They have found out a SNP in the gene coding SLCO1B1 (Soluble Carrier Organic Anion Transporter) is responsible for the increase in the risk for myalgia [1]. When the transporter is modified by the variant allele the statins are not properly transported across the hepatocytes. They are not metabolized and stay in the body for longer time and cause myalgia. The mechanism by which statins produce myalgia is uncertain. Various theories are put forward. Though all drugs belonging to this class have a tendency to produce myalgia the degree varies for different drugs.

Minor increases in creatine kinase (CK) activity are observed in myalgia. It is associated with heavy physical activity. In rare cases myopathy occurs (1 in 1000.), where there is marked elevations in CK activity, often accompanied by generalized discomfort or weakness in skeletal muscles. In very severe cases it may proceed to rhabdomyolysis. Few deaths have been reported. If the drug is not discontinued, myoglobinuria can occur, leading to renal injury.

Measurement of Creatine kinase helps to grade the severity of myalgia. It acts as a surrogate marker. There are 3 isoforms of the enzyme CK (MM). CK(MB) and CK(BB). The isoform pertaining to muscle injury is CK(MM). The normal range of CK(MM) is 0-24 IU/L.

SLCO1B1 Gene

It is a gene encoding for solute carrier organic anion transporter family member 1B1. It is located the short (p) arm of chromosome 12 at position12.1 [2].

Polymorphisms in SLCO1B1 Gene

There are 14 non-synonymous SNPs, represented by 16 distinct haplotypes, named SLCO1B1*1 to SLCO1B1*14 (reference haplotype = SLCO1B1*1a). Since then, a further haplotype, **15,* has also been identified. Only c.388A>G (rs2306283) and c.521T>C (rs4149056) are associated with altered transport function [1]. A common polymorphism in SLCO1B1 gene is rs4149056. It results due to change of the amino acid valine with the amino acid alanine at position 174 in the transporter protein. It is written as V174A or *SLCO1B1**5 [3].

The different genotypes of SLCO1B1*5 are

T/T genotype (valine/valine): Normal
T/C genotype (valine/alanine). Heterozygous
C/C genotype (alanine/alanine): Homozygous

In a study by SEARCH Collaborative Group, it was observed that the homozygotes of variant allele (CC) had an 18% cumulative risk, with myopathy occurring primarily during the first year, whereas the heterozygous (CT) genotype was associated with a cumulative risk of about 3%. In contrast, the cumulative risk of myopathy was only 0.6% among TT homozygotes [4].

In a study done in The University of Wisconsin Hospital it was found that the mean duration of statin therapy before onset of myopathy was 6.3 months. Resolution of muscle pain occurred 2.3 months after discontinuation of statin therapy [5].

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METHODOLOGY

Ethical Clearance

Institutional Ethical committee of Madurai Medical College, Madurai approved the proposal to conduct this study in the Department of Cardiology and Department of Medicine for myalgia induced by statins.

Aim

To determine the prevalence of single nucleotide polymorphism (SNP)- SLCO1B1* 5 allele and its association with myopathy due to atorvastatin.

Inclusion Criteria

- Patients who were on atorvastatin for 2 months and more.

Exclusion Criteria

- Patients with the history of myopathy and any other myalgias before starting the drug
- Patients on drugs which also tend to produce myalgia like fibric acid derivatives, antifungals
- Patients who received blood within 6 months
- Patients unwilling to undergo the study
- Renal or hepatic insufficiency

Cases

Patients on statin found to be positive for SLCO1B1*5 allele

Controls

Patients on statins found to be negative for SLCO1B1*5 allele.

Sample size

194

Sample size

The incidence of C Allele the variant in SLCO1B1 gene found to be 5-10% in South Asians [6]. In a study done by Stewart, the Odds ratio for association between SLCO1B1*5 and myopathy in patients taking atorvastatin at a dose of 20-40 mg was found to be 2.7 [6]. Computing these values in epi.info the sample size obtained was 194.

Methodology

Patients on treatment with statins were selected after explaining the nature of the study and getting an informed consent. They were selected by convenient sampling method. Those who were willing to participate were included in the study. The details of the patient like age, sex, and duration of drug intake were recorded. All of them were enquired specifically for the signs of myalgia. The diagnostic criteria of myalgia are based on the Canadian Working Group Consensus. After getting written consent, 5ml blood was withdrawn and collected in ethylene diamine tetra acetic acid-coated sterile vials and stored in a refrigerator

at 4°C. The blood samples were sent to the Immunology department of Madurai Kamaraj University and tested for the presence of SNP in SLCO1B1 gene. And simultaneously levels of CPK (MM) were also evaluated.

The patients on atorvastatin are divided into a low dose group (receiving 10-20mg) and a high dose group (receiving 40-80mg).The samples were collected in medicine OPD and cardiology review OPD. In medicine department atorvastatin is prescribed for hyperlipidaemic patients, diabetics and chronic renal failure patients. They are usually given 10-20mg/day. In cardiology, patients with ischaemic heart disease who underwent percutaneous coronary intervention were given 80mg/day for 2 months and the dose is gradually decreased every 2 months to 60mg, 40mg and 20 mg/day respectively

After DNA extraction the presence of SNP in SLCO1B1*5 was tested by polymerase chain reaction with sequence specific primers. DNA was extracted based on salting out method (Miller et al., 1988) [7]. The detection of SLCO1B1 genotyping was performed as described by Zhang et al., 2006 [8] using sequences specific primers.

All the patients are periodically reviewed about the development of symptoms of myalgia.

Statistical analysis

All variables were analyzed descriptively. For the continuous variables, data were expressed as the mean ± standard deviation (SD). Student's t-test and ANOVA with post hoc Tukey were performed for variables with a normal distribution. The Fishers exact test was performed for categorical variables. Odds ratios (ORs) were calculated. Statistical analyses were performed using the epi.info

RESULTS

A total of 192 blood samples were collected with 96 samples in each group. In low dose group the number of males was 55 and females were 41. The average age of the patients is 55. The range is from 32-73. In high dose group there were 79 males and 17 females. The average age was 59 with the range from 39 to 85.

The blood samples on testing for SLCO1B1 *5 yielded the following results.

The Wild homozygote (TT) was observed in 72 patients; the heterozygous variant (CT) was observed in 106 patients; the homozygous variant (CC) was rare, seen in 14 patients.

The prevalence of T allele is 65% and C allele is 35%.

When enquired about the adverse effects only 32 patients complained of myalgia. The presence of myalgia in three genotypes is given in Table 1 and Figure 1.

Figure 1: Prevalence of Myalgia in Different Genotypes.

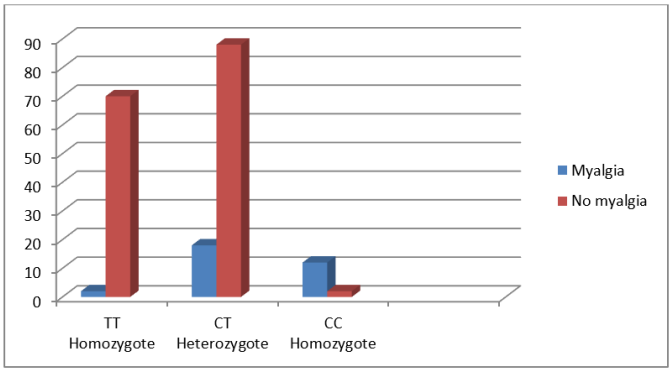

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Table 1: Prevalence of Myalgia in Different Genotypes.

Genotypes	Myalgia	No Myalgia
TT Homozygote	2	70
CT Heterozygote	18	88
CC Homozygote	12	2

On applying fishers exact test for normal and heterozygous individuals p=.0030. The result is significant at $p < 0.01$. On applying fishers exact test for normal and homozygous individuals p<.0001. The result is highly significant. So, the incidence of myalgia is more with variant allele. Homozygous patient suffers more than heterozygous patients

The incidence of myalgia between low dose and high dose are given in Table 2.

Table 2: Prevalence of Myalgia with the Two Dose Level.

Dose level	TT Homozygotes		CT Heterozygotes		CC Homozygotes	
	Myalgia	No Myalgia	Myalgia	No Myalgia	Myalgia	No Myalgia
Low Dose	1	24	4	59	6	2
High Dose	1	46	14	29	6	0

Using Fishers exact test in TT Homozygous individuals, the higher dose does not increase the risk for myalgia in TT Homozygotes individuals. In CT heterozygous individuals p= .0010. It is highly significant at p<.05.The higher dose increase the risk for myalgia in heterozygous individuals. In CC homozygous individuals p=.4725. It is not significant at p<.05. In CC homozygous individuals the risk for myopathy is more even at a lower dose.

The levels of creatinine kinase increase with myopathy associated with statin. The CPK MM level taken after 8 weeks of the drug intake was tabulated as shown in Table 3.

Table 3: Average CPK MM Level between Patients with Myalgia and Tolerant Individuals

Genetic status	Low dose level		High Dose level	
	Myalgia	No Myalgia	Myalgia	No Myalgia
TT	17.28±0	10.29±2.98	39.38±0	18.21±4.64
CT	35.9±14.25	14.60 ±4.7	56.22±20.70	20.64±4.25
CC	128.03±41.44	48.37±2.55	192.19±25.02	Nil

By using Student's t-test there is a significant difference in CPK MM level between patient with myalgia and tolerant individuals in CT heterozygous and CC homozygous genotypes. The CPK MM level is increased more with higher dose

DISCUSSION

The genetic study revealed that the variant allele C is commonly prevalent in our population. The wild allele T is present in 65% of the study population and the variant C allele in 35% of the study group. The C allele presents as 55% CT heterozygotes and 7% CC homozygotes. In a previous study it was observed the frequency of C allele in South/West Asia is 5-10% [5].

Among the heterozygous group 6% of persons in low dose group and 32% patients in high dose group complained of myalgia. The CPK MM level is elevated to 1-2 times of ULN in patients with myalgia in low dose group, and 1-3 times of ULN in high dose group. As per definition it is considered as mild Grade 1 elevation of CPK MM.

Among the CC homozygous individuals 75% persons in low dose level and 100% persons in high dose level had myalgia. The CPK level is increased by 2-8 times above upper limit in low dose level and 6-9 times above upper limit in high dose group. It ranges from Mild Grade 1 & Grade 2 elevation.

As no patient had CPK level more than 10 times the upper limit they can be considered as mild myopathy.

In TT homozygotes one person each complains of myalgia. In low dose level there is no elevation of CPK MM. In higher dose it was mildly elevated.

Hence it is evident the presence of C allele increases the risk of myopathy and it is well observed in the CC homozygous individuals. It is also evident that increase in dose increases the risk for myopathy.

In a genome-wide study done by Search Collaborative Study group it was found that the odds ratio for myopathy was 4.5 (95% confidence interval [CI], 2.6 to 7.7) per copy of the C allele, and 16.9 (95% CI, 4.7 to 61.1) in CC as compared with TT homozygotes [4]. More than 60% of these myopathy cases could be attributed to the C variant. They have done the study in patients taking 80mg simvastatin for 8 weeks.

In a meta-analysis by Turongkaravee S, Jittikoon J, Lukkunaprasit T et.al, it was found that CC and TC genotypes suggested a higher risk of myopathy in atorvastatin users [OR = 4.0 (1.23, 12.63) and OR = 2.0 (1.11, 3.52), respectively] than those who carried TT genotype in a pooled data [9]. In the studies included in the meta-analysis the dose of atorvastatin was between 20-80mg.

In this study the odds ratio for CT heterozygotes in lower dose is 1.5873(95%CI 0.1690-14.9069) as compared to TT homozygotes ; the odds ratio for CC homozygotes in lower dose is 18.75 (95%CI 1.95-180) as compared to TT homozygotes.

The odds ratio for CT in higher dose is 12.80 (95%CI 1.61-101.5) as compared to TT homozygotes; the odds ratio for CC in higher dose is 43 (95%CI 4.38-421.6) as compared to TT homozygotes.

In a study by Kennedy SP, Barnas GP,et al it is stated that as both atorvastatin and rosuvastatin have long half-lives, non-daily dosing may reduce adverse effects while still achieving adequate reduction of LDL-c [10]. In a study done by Matalka MS, Ravnan MC et al., it was stated that alternate-day administration of atorvastatin can produce a reduction in LDL-C comparable to that of daily administration in patients with hypercholesterolemia [11]. In a study on statin-related myopathy by Joy TR, Hegele RA. it is stated that the options for managing statin myopathy include statin switching, particularly to fluvastatin or low-dose rosuvastatin; non-daily dosing regimens; nonstatin alternatives, such as ezetimibe and bile acid–binding resins; and coenzyme Q10 supplementation [12].

These methods can be followed in an individual with variant C allele and complains of severe myalgia to control lipid levels.

The reason proposed for myalgia is statins reduce the levels of coenzyme Q10 (CoQ10) in muscle and also alters the normal fatty acid metabolism in myocytes leading to muscle damage. It also increases levels of ATROGIN1, which is involved in ubiquitination, leading to increased muscle protein degradation [13].

SUMMARY

The genetic variant SLCO1B1*5 is widely prevalent in the local population. There is increased risk of developing myalgia with statin in CC homozygous individuals and to a lesser extent in CT heterozygous individuals. But the increase in CPK MM is only of mild grade. So it need not be a mandatory to check genetic status before starting statins. But if a patient complains of myalgia serum CPK MM level can be measured and if it is raised to a greater extent genetic testing can be done. Prescribing an alternate statin or reduction in the dose can be followed to reduce the myalgia and thereby increasing the compliance which will help to achieve the therapeutic goal.

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