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The lipid-lowering efficacy of rosuvastatin is associated with variations in *SLCO1B1*: a 12-month prospective cohort study

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Abstract. – OBJECTIVE: Statins' efficacy and safety are subject to wide inter-individual variability, partly due to genetic predisposition. Studies have shown that the genetic variations in the common solute carrier organic anion transporter family member 1B1 *(SLCO1B1)* gene polymorphisms affect the transport of statins' transport into hepatocytes, their plasma concentration, and circulation time. The ultimate result is variable and personalized statins response and statin-associated muscular symptoms (SAMS). Here we report an update on the differential response to rosuvastatin therapy in the Pakistani population.

PATIENTS AND METHODS: A total of 166 hyperlipidemic patients on rosuvastatin were prospectively followed for 24 weeks. Muscle symptoms were recorded after 6-8 weeks of therapy, and assessment was done according to the SAMS-clinical index tool. Patients were genotyped for *SLCO1B1* c.521T>C and c.388A>G polymorphisms, for association with lipid-lowering response and statin-associated muscle symptoms. The plasma level of rosuvastatin was determined through Liquid chromatography-mass spectrometry (LCMS) for possible correlation with adverse effects and lipid-lowering efficacy.

RESULTS: Mean reduction in low-density lipoprotein cholesterol (LDL-C) was 42.34 mg/dl (p<0.001), 35.66 mg/dl (p<0.001), and 24.47 mg/dl (p=0.202) in reference, heterozygous and mutant homozygous groups of *SLCO1B1* c.521T>C, respectively. A 15.70% and 42.14% diminished LDL-C reduction was observed in c.521TC and c.521CC, respectively, compared to the reference c.521TT genotype. Similarly, for *SLCO1B1* c.388A>G, 20.50% and 29.40% less LDL-C lowering effect was observed in heterozygous and mutant homozygous carriers, respectively. SAMS were observed in 37% and 33% of heterozygous and minor homozygous, respectively, (p=0.059). The rosuvastatin plasma level was 1.89-fold higher in the c.521CC genotype than in the reference homozygous type.

CONCLUSIONS: Differential lipid-lowering response and muscular symptoms due to rosuvastatin are associated with the *SLCO1B1* common polymorphisms. Further studies are needed to validate dose adjustment and rationalization.

Key Words:
Coronary artery disease (CAD), Dyslipidemia, Statins, SLCO1B1.

Introduction

Statins are the first-line, widely prescribed[1] lipid-lowering agents for managing dyslipidemia in primary and secondary prevention of cardiovascular diseases (CVD)[2]. The 3-hydroxy 3-methyl-glutaryl coenzyme A reductase inhibitors (statins) competitively block the rate-limiting step in the *de novo* synthesis of cholesterol. This inhibition further leads to upregulation of the low-density lipoprotein receptors on the hepatocytes' cell surface. More low-density lipoprotein cholesterol (LDL-C) is cleared from the bloodstream[3], causing a 20-50% reduction in plasma LDL-C levels[4]. In a log-linear way, a 1% decrease in the LDL-C levels is associated with a 1% risk reduction in major CVD events[5]. The current guidelines for preventing coronary artery disease (CAD) risk consider LDL-C reduction as the primary target[5,6]. Statins also exert cholesterol-independent, pleiotropic effects by decreasing inflammatory mediators and improving vascular endothelial function[7]. In a recently published study[8], statins were found to suppress proinflammatory cytokines, CCL11, CSF2, CCL20, and TGFB1 (p<0.05) in TNF-alpha-treated cells derived from human saphenous veins.

Despite its broad use in CVDs, statins are reported to have considerably variable lipid-lowering effi-

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cacy and musculoskeletal symptoms[9]. Inter-ethnic and inter-individual variability has been reported to affect up to 30-50% of the treated patients, failing to achieve the optimal lipid-lowering goals. Variable lipid-lowering response of statins has been documented in association with the variations in c.521T>C[10]. The plasma level of rosuvastatin has been reported to be 1.4-fold and 2.2-fold high in heterozygous and homozygous mutant carriers of the *SLCO1B1* c.521T>C compared to the reference genotype[11]. The Statin-Associated Muscle Symptoms (SAMS) affect as high as 10-25% of statin users[12]. SLCO1B1 c.521T>C and c.388A>G have been reported to be associated with SAMS in genome-wide association studies (GWAS)[13-16] and meta-analyses[17,18].

Since statins are prescribed for a longer duration, nearly half of the patients are reported to have stopped the treatment after one year, mainly due to muscle pain, possibly due to differential genotypic presentation[19]. The current study investigated the association of differential lipid-lowering response and muscular adverse effects of rosuvastatin with single nucleotide polymorphisms in the *SLCO1B1* gene in hyperlipidemic patients of the northwestern Pakistani population.

Patients and Methods

Study Design

In this prospective cohort study, hyperlipidemic patients with CAD were enrolled and followed from February 2019 to March 2020. The study design was approved by Khyber Medical University Advance Studies and Research Board (KMU-AS&RB). Ethical approval was obtained from Khyber Medical University Ethical Review Board No. DIR/KMU/EB/AL/000518 and Medical Teaching Institute-Hayatabad Medical Complex (MTI-HMC) Peshawar ref. No. 059/HEC/PICO/18.

Study Settings and Patients

In total, 166 patients of either sex were enrolled from Medical Teaching Instititue-Hayatabad Medical Complex (MTI-HMC) Peshawar and district headquarter hospitals of Malakand and Parachinar districts of Khyber Pakhtunkhwa province of Pakistan. Patients were enrolled after signing a written informed consent form. Of the 166 patients, 35 participants were lost during followup, and 131 participants' data were finally analyzed. CAD Patients on rosuvastatin 10 mg treatment were asked to participate if their total cholesterol (TC) was 160 mg/dl or above. Patients were excluded from the study if they had (i) thyroid dysregulation, chronic kidney disease, uncontrolled diabetes mellitus or blood pressure, or (ii) a history of significant change in diet or weight in the previous month.

Data Acquisition and Samples Collection

Purposefully designed and validated data acquisition forms for patient information were used to collect demographic, anthropometric, and clinical data at baseline and during follow-up visits. A 5 ml blood was collected and processed accordingly for (i) lipid profile, (ii) drug plasma level, and (iii) DNA extraction in respective visits.

DNA Extraction and Amplification

DNA was extracted through a modified salting-out technique from etheylenediaminetetraacetic acid (EDTA)-anticoagulated blood[20]. The following sequencing primers were used for amplification and Sanger sequencing. For *SLCO1B1* rs4149056 (c.521T>C) as: (Forward) 5'-ACCATATTGTCAAAGTTTGCAAAGTGA-3', and (Reverse) 5'-TTCAAAAGTAGACAAAGGGAAAGTGATC-3'. For *SLCO1B1* rs2306283 (c.388A>G), forward 5'-GACTGATCATCTTTGAAGAT-3', and reverse, 5'- ATTAACACTATAATTATGTC -3'primers were used

Sanger Sequencing

Targeted sequencing of the *SLCO1B1* for c.521T>C and c.388A>G was performed on SeqStudio™ genetic analyzer (Applied Biosystems, Waltham, Massachusetts, USA). Amplified PCR products with amplicon sizes of 358 bp and 635 bp were cycle-sequenced with a BDT v.3.1 (Applied Biosystems, Waltham, Massachusetts, USA) master mix as per the manufacturer's instructions for forward and reverse primers. BigDye XTerminator™ (Applied Biosystems, Waltham, Massachusetts, USA) kit was used for the purification of cycle-sequenced products. Samples were loaded to the SeqStudio™ genetic analyzer, and run parameters were set according to the kits used. The sequence data in the electropherogram was analyzed with Finch TV™ (v 1.4, Geospiza Inc., Seattle, WA, USA) genetic software, and SNP locations were visually checked for polymorphisms.

Lipid Profile Determination

Total cholesterol, high-density lipoprotein cholesterol, triglycerides, and low-density lipoprotein cholesterol were determined using different kits compatible with Cobas c111 biochemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Statin-Associated Muscle Symptoms Assessment

SAMS clinical Index (SAMS-CI) tool was used as, previously reported, by recording the location, symmetry, and onset of muscle symptoms after 6-8 weeks of rosuvastatin therapy. Patients reporting the presence of muscular pain were further divided into "Unlikely", "Possible", and "Probable" categories based on their SAMS-CI score[21].

Plasma Level of Rosuvastatin

Rosuvastatin plasma concentration was determined at a steady state by Liquid Chromatography coupled with mass-spectrometry (LC-MS/MS). Serum samples taken after 6-8 weeks of rosuvastatin therapy were analyzed through LC-MS/MS, with a modified analytical method, as published earlier by our group[22].

Statistical Analysis

The frequencies, percentages, means, and standard deviations were used as descriptive statistics. All statistical analyses were performed using SPSS software (version 22; IBM Corp., Armonk, NY, USA). Differences between numerical variables were computed using Paired sample *t*-test, and the Chi-square test (χ^2) or Fisher's exact test, where appropriate, was used to determine the association between categorical variables. Hardy-Weinberg Equilibrium (HWE) was determined using the χ^2 test. A *p*-value greater than 0.05 indicated that the observed genotype distributions were consistent with HWE assumptions. The one-way ANOVA was used for continuous variables with more than two groups. A statistically significant association for all tests was reported at a p-value <0.05.

Results

Background Characteristics

The mean age of the study participants was 53.46±9.94 years, ranging from 30 to 76 years. Majority of the patients. n=96 (57.83%) were in the late middle age group (46-60 years), with 51.2% (n=85) males. Similarly, the mean body mass index (BMI) was 26.99±2.43 kg/m^2. The majority n=108 (65.10%) of the participants were overweight according to BMI cut-offs as defined by World Health Organization (WHO), while obese n=133 (81.10%) based on BMI cut-offs for Asians. Diabetes mellitus (DM) was the most prevalent comorbid condition. The proportion of current smokers was 9.6%, as shown in Table I.

Table I. Background characteristics of the study population.

Characteristics		Mean±SD	N	%
Age		53.46±9.95		
Age groups	30-45 years		34	20.48
	46-60 years		96	57.83
	61-76 years		36	21.69
Gender	Female		81	48.80
	Male		85	51.20
Weight Kgs		77.95±7.56		
Height (cm)		169.98±6.05		
Body mass index		26.99±2.44		
BMI WHO cutoffs	Normal		37	22.29
	Over Weight		108	65.06
	Obese		21	12.65
BMI Asian cutoffs	Normal		4	2.41
	Over Weight		29	17.47
	Obese		133	80.12
Current smoking	Non-Smokers		150	90.36
	Current Smokers		16	9.64
Diabetes mellitus	Non-diabetic		137	82.53
	Diabetic		29	17.47
Coronary artery disease intervention	No		136	81.93
	Yes		30	18.07

Table II. Genotypes and Alleles frequencies of c.521T>C and c.388A>G polymorphisms.

Genes and polymorphisms	Genotypes	Frequency (%)	Alleles	Frequency (%)	*p*-value	HWE χ^2
SLCO1B1 c.521T>C	TT	93 (82.3)	C	87.36	0.16	3.54
(rs4149056)	CT	17 (15.04)	T	12.64		
	CC	3 (2.65)				
SLCO1B1 c.388A>G	AA	46 (36.8)	A	58.8	0.59	1.055
(rs2306283)	AG	55 (44)	G	41.2		
	GG	24 (19.2)				

Genotypes and Alleles Distribution of c.521T>C and c.388A>G

It was observed that 82.30% (n=93) and 44% (n=55) of the patients had the wild *SLCO1B1* c.521T>C and heterozygous mutant *SLCO1B1* c.521T>C genotypes. The minor allele frequency was 12.64% and 41.2% for c.521T and c.388G, respectively. The observed frequencies were in agreement with the Hardy-Weinberg Equilibrium ($p>0.05$), as shown in Table II.

Mean Changes in the Plasma Lipids

After 24 weeks of rosuvastatin therapy, plasma TC, HDL-C, TG, and LDL-C were significantly improved in our cohort. For the *SLCO1B1* c.521TT genotype, there was a reduction in TC, TG, and LDL-C, while an increase in HDL-C was statistically significant ($p<0.001$). In the c.521TC heterozygous patients, the mean increase in HDL-C and decrease in TG were not statistically significant ($p=0.132$ and 0.153, respectively). In the minor homozygous genotypes, none of the lipid parameters changed significantly ($p\geq0.05$), as shown in Table III. Similarly, for c.388AG, the change in TC, HDL-C, TG, and LDL-C was significant after the treatment in all genotypes, except for the increase in HDL-C in the heterozygous (AG) genotype, where the change was statistically insignificant ($p=0.419$) (Table III). LDL-C, the primary target of statin therapy, decreased by a mean of 34.15 mg/dl in overall study participants. Among patients with SLCO1B1 c.521T>CC polymorphism, the mean reduction in plasma LDL-C was 42 mg/dl, 35.66 mg/dl, and 24.47 mg/dl in normal homozygous, heterozygous, and homo mutant genotypes, respectively.

Individual Lipid-Lowering Response

Patients were deemed responders if their LDL-C reduction was greater than 10% or non-responders in case of less than 10% LDL-C decline. Treatment failure was noted in 12.20% (n=16) after 24 weeks of rosuvastatin treatment (Figure 1). In the responder group, the reduction in LDL-C ranged from 11-63%.

Plasma Level of Rosuvastatin

The mean plasma concentration in the homozygous (TT) group was 6.14±4.00 ng/ml, while for the heterozygous (TC) was 7.48±4.68 ng/ml and for the homozygous (CC) was 11.66±5.63 ng/ml as shown in Table IV. The mean plasma level in the homozygous (CC) was 189% higher than the homozygous wild type (TT). however, no statistically significant difference ($p=0.057$) was observed, as shown in Table IV. In case of c.388A>G, mean plasma level among reference genotypes was 5.29±2.65 ng/ml, in the (AG), it was 7.45±4.97 ng/ml and for mutant homozygous (GG), it was 5.60±4.27 ng/ml as shown in Table IV. However, no statistically significant difference was observed.

Statins-Associated Muscle Symptoms

A total of n=31 (23.66%) participants reported muscular symptoms based on the interviews and assessment of the muscular complaints. Muscular symptoms were reported in 37.5% (n=6) of the c.521TC genotypes, compared to 22% in the normal genotypes. Similarly, in the homo mutant genotypes, the ratio of muscular symptoms was 33.33%, as shown in Table V.

After the application of the SAMS-CI tool, n=7 (22%) patients were found to be "unlikely" to have SAMS, while n=14 (45.16%) patients were in the "Possible" category, and n=10 (32.25%) were in the "Probable" category of muscular symptoms on the SAMS-CI scale as shown in Figure 2.

Table III. Mean change in total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, and low-density lipoprotein cholesterol (LDL-C) with respective polymorphisms of *SLCO1B1* gene.

	1 c.521T>C								
	TT			TC			CC		
Lipids	Baseline	Follow-up	*p*-value	Baseline	Follow-up	*p*-value	Baseline	Follow-up	*p*-value
TC	213.51±28.51	166.87±20.14	<0.001*	207.88±33.91	168.19±24.16	<0.001*	233.33±53.91	200.33±46.76	0.223
HDL-C	36.78±6.26	38.63±3.95	0.001*	37.50±7.78	38.94±5.30	0.132	41.33±11.85	39.67±5.03	0.742
TG	222.32±69.01	191.57±53.08	<0.001*	224.13±57.00	201.06±69.83	0.153	270.67±75.04	236.33±59.53	0.141
LDL-C	132.27±23.17	89.93±20.67	<0.001*	125.55±32.55	89.89±31.36	<0.001*	137.87±32.99	113.40±32.24	0.202
	c.388A>G								
	AA			AG			GG		
Lipids	Baseline	Follow-up	*p*-value	Baseline	Follow-up	*p*-value	Baseline	Follow-up	*p*-value
TTC	213.51±24.94	170.94±26.45	<0.001*	213.46±33.93	169.76±21.45	<0.001*	203.38±28.05	164.75±23.86	<0.001*
HDL-C	37.03±7.08	39.50±4.88	<0.001*	37.55±6.67	38.11±3.92	0.419	35.94±4.63	38.81±3.39	0.001*
TGs	222.32±69.01	196.00±63.25	0.050*	228.86±75.02	195.36±58.90	0.001*	227.95±61.51	187.44±36.41	0.010*
LDL-C	139.53±22.89	92.24±28.56	<0.001*	130.14±27.09	92.56±19.74	<0.001*	121.85±22.58	88.45±19.02	<0.001*

Paired *t*-test was performed, **p*-value <0.05 statistically significant.

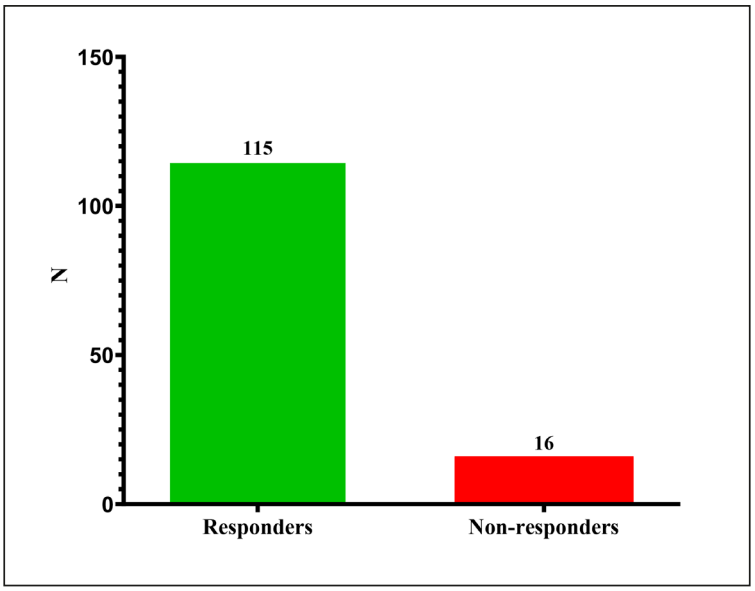

Figure 1. Responder *vs.* non-responders in the study participants.

Table IV. Data of study groups and ıntergroup significance value.

Gene		Plasma level of rosuvastatin						*p*-value
		Mean	SD	Median	Range	Minimum	Maximum	
SLCO1B1 c.521T>C	TT	6.14	4	5.77	22.71	1.20	21.77	0.057
	TC	7.48	4.68	6.86	18.41	2.01	20.42	
	CC	11.66	5.63	14.86	9.81	5.15	14.96	
SLCO1B1 c.388A>G	AA	5.29	2.65	5.62	10.32	1.06	9.39	0.066
	AG	7.45	4.97	6.86	22.71	1.27	21.77	
	GG	5.6	4.27	5.16	14.48	0.38	14.86	

One-way ANOVA; *p*-value <0.05 statistically significant.

Table V. Association of the muscular symptoms (presence or absence) with SLCO1B1 polymorphisms.

SAMS	SLCO1B1 c.521T>C			SLCO1B1 c.388A>G		
	TT N (%)	TC N (%)	CC N (%)	AA N (%)	AG N (%)	GG N (%)
Absence	70 (77.8)	10 (62.5)	2(66.7)	33 (76.6)	40 (74.1)	17 (77.3)
Presence	20 (22.2)	6 (37.5)	1(33.3)	10 (23.3)	14 (25.9)	5 (22.7)
p-value		0.291			0.96	

Fisher's exact test was used; *p*-value <0.05 significant.

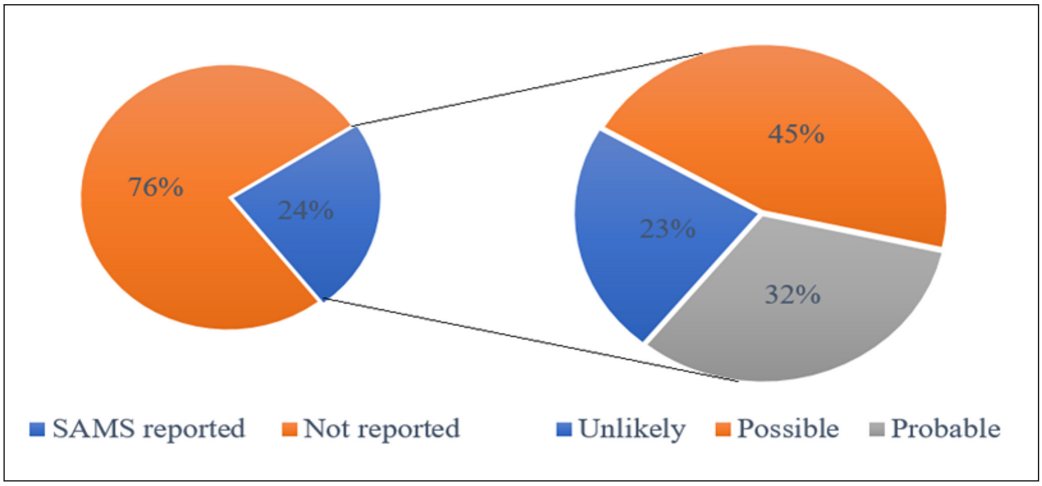

Figure 2. Distribution of Muscular Symptoms based on SAMS-CI tool. Out of the 31, n=7 (22%) patients were classified as "unlikely" to have SAMS, n=14 (45.16%) patients were in the "Possible" category, and n=10 (32.25%) were in the "Probable" category of muscular symptoms."

Discussion

Variability in statins' lipid-lowering efficacy and associated muscle symptoms are partly due to genetic pre-disposition and are widely reported[15,23,24]. The current study investigated the association of c.521T>C and c.388A>G polymorphisms in the hepatic influx transporter encoding gene *SLCO1B1* with lipid-lowering efficacy and safety of rosuvastatin.

In our cohort, the lipid-lowering response was significant, with a mean 34.15±14.17 mg/dl reduction in LDL-C in all age groups, without any significant differences based on background characteristics. In association with the *SLCO1B1* genotypes, a remarkable improvement in all lipid parameters was noted in the wild c.521T/T genotype. At the same time, 15.70% and 42.14% diminished LDL-C reduction was observed in *SLCO1B1* c.521TC and c.521CC, respectively, compared to the normal genotype. Similar observations were reported by Tachibana et al[25] in the Japanese population, with a 22% reduction in the T/T *vs.* 16% in T/C genotypes, suggesting a modulation of the lipid-lowering efficacy by the c.521T>C polymorphisms[25]. The *SLCO1B1**5 haplotype has consistently been reported with a decreased lipid-lowering effect, showing a 19.3% reduction in LDL-C compared to 32.2% in wildtype[10]. The second polymorphic genetic marker in the study, *SLCO1B1* c.388A>G, had no prominent effect on rosuvastatin's efficacy', and the LDL-C reduction was significant in all patients except for the HDL-C in the heterozygous genotype (p=0.149). The plasma level of rosuvastatin was found to be statistically insignificant though noticeably variable in the different genotypes of the OATP1B1 phenotype. For the c.521CC, the plasma level of rosuvastatin was 2-fold, while in c.388AG, it was 1.4-fold higher than the respective reference genotypes. Similar results for high plasma levels of rosuvastatin were reported by Wagner et al[11] with a mean plasma level of 10.10 ng/ml in the CC genotype compared to 4.30 ng/ml in the reference homozygous allele[11]. Congruent observations were also reported by Tirona et al[26] with up to 117% higher rosuvastatin plasma concentration in the c.521CC genotypes[26]. Higher plasma levels are reported consistently in the mutant genotypes of c.521T>C, making it a strong candidate gene for genotype-guided personalized therapy. Mechanistic explanations suggest a decrease in the function of the OATP1B1 phenotype as predicted by the *SLCO1B1* variant genotype as reported by Richard et al[27]. Due to elevated plasma concentration of statins in Asians, a lower initial dose of 5 mg rosuvastatin has been recommended by the US food and drug administration (FDA)[28]. There was a noticeable difference in the plasma level of c.388A>G heterozygous and minor homozygous genotypes, with a mean plasma concentration of 7.45 ng/ml and 5.6 ng/ml, respectively, compared to the normal genotype (AA) at 5.29 ng/ml. Concordant to our observations, Lehtisalo et al[29] have also reported a 2.2-fold high plasma concentration of rosuvastatin in the heterozygotes compared to the normal genotypes.

In total, 23.66% (n=31) of the patients reported muscular pain with statin therapy. With a further assessment of the symptoms for pain location, symmetry, onset, and resolving after rosuvastatin discontinuation, patients were assigned to "unlikely, possible, and probable" categories for statin-associated muscle symptoms. Out of the total patients with reported symptoms (n=31), 23% (n=7) were unlikely for the statin-associated muscle symptoms based on their SAMS-CI score, while 45% (n=14) and 32% (n=10) were in the "possible" and "probable" categories, respectively for having muscular pain as a result of statins therapy. Muscular symptoms with statins are widely reported [30-32]. In a study published in 2017, Taylor et al. [33] reported the prevalence of SAMS in 38% of statin users, as confirmed by a crossover protocol with simvastatin and placebo. Statins pharmacogenetics studies[18,28] have mainly focused on the SAMS, as the *SLCO1B1* c.521C allele has been reported in several studies[13-18], including GWAS. A recently published study[34] on *SLCO1B1*'s association with SAMS reported that carriers of the T521C allele had a higher risk for neuromuscular pain. In our study, the association was not statistically significant. Albeit, this can partly be explained by the very low sample size in our study as only n=1 patients reported the muscular symptoms in the homozygous mutant group. Upon detailed interviews for the pain location, symmetry, and patients with low scores mentioned backache or headache but not muscular pain.

Limitations

Due to low socio-economic status and less developed healthcare record systems, follow-up of patients was not ideal. Loss to follow-up was common, without consultation with the physicians. This behaviour limited the chances of recording the reasons for dropping out. As multiple genes are involved in the lipids and statin transport and metabolisms pathways; we investigated only one gene due to limited resources for genetic testing. Also, the study's sample size was too small to cover the serious but rare muscular adverse events. Due to the low sample size, the minor allele carriers were very few in the cohort, limiting the chances of establishing a significant correlation with lipid-lowering efficacy. Patients were followed only for 24 weeks, so long-term response and adverse effects could not be observed.

Conclusions

c.521TC and c.388AG polymorphisms in the *SLCO1B1* gene have a notable association with lipid-lowering response and muscular adverse effects due to rosuvastatin therapy in the Pakistani population, and play an essential role in the termination of the therapy by patients without consultation with the physician.

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Ethics Approval

Ethical approval was obtained from Khyber Medical University Ethical Review Board vide letter No. DIR/KMU/EB/AL/000518 and Medical Teaching Institute Hayatabad Medical Complex (MTI-HMC) Peshawar ref. No. 059/HEC/PICO/18.

Informed Consent

A written informed consent form designed in the local language, was given to the candidate participants, and explained to them. After they willfully agreed to participate in the study, they were requested to put their signature on the document.

Authors' Contributions

Muhammad Zakria Arif Hussain, and Sami Siraj: conceptualization, data collection, manuscript writing, literature review, and data checking; Arif Hussain, Nasir Ahmad, and Muhammad Abdur Rauf helped in clinical observations and data collection. Naseer Ahmad: Critical reviewing, figures correction, and proofreading. All authors have read and approved the manuscript.

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Conflict of Interest

The Authors declare that they have no conflict of interest.

Data Availability

Datasets generated during the current study have not been uploaded to any repository and are not available online, because there are still unpublished parameters in the combined dataset, and more publications will be made in near future. But the data analyzed are available from the corresponding author on reasonable request.

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