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Effect of ABCA1-R219K Polymorphism in Serum Lipid Parameters in Patients under Statin Therapy Visiting Tertiary Cardiac Center of Nepal

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Abstract

Introduction ATP-binding cassette transporter A1 (ABCA1) encoded by ABCA1 gene is one of the important protein involved in lipid metabolism. The effect of statin therapy on dyslipidemia varies among individuals and it may be due to different genetic polymorphism. The R219K polymorphism of ABCA1 gene is found to have a significant role in the response of statin.

Objective This study was designed to evaluate the effect of R219K polymorphism in lipid-lowering action of statin in patients with dyslipidemia.

Material and Methods This study was conducted in 88 patients. Blood samples were taken from patients before and at the end of 3 months of statin use and were analyzed for lipid profile. Whole blood was analyzed for R219K Polymorphism using polymerase chain reaction-restriction fragment length polymorphism.

Results R219K polymorphism was associated with significant percentage reduction of serum triglyceride/high-density lipoprotein (TG/HDL) ratio and total cholesterol/high-density lipoprotein (TC/HDL) ratio in atorvastatin users. However, there was no significant association of polymorphism with change in serum TC, HDL-C, LDL-C, TG, and very low-density lipoprotein (VLDL). Among KK genotype individuals, value of TG, VLDL, TG/HDL, and TC/HDL were significantly lower than in RR genotypes. Also, TG/HDL and TC/HDL were significantly lower in RK genotype than in RR. Treatment of

Keywords
- R219K polymorphism
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- single-nucleotide polymorphisms
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- statin therapy

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dyslipidemia with statin was found to be comparatively better in patients having the genotypes KK and RK.

Conclusion Our study demonstrated association of R219K polymorphism with the significant reduction of TG/HDL and TC/HDL and particularly the KK genotype was associated with significant improvement of lipid parameters following atorvastatin treatment.

Introduction

Dyslipidemia is the principle metabolic comorbidities which is associated with excessive body fat like increased level of triglycerides (TG) or low-density lipoprotein-cholesterol (LDL-C), or decreased high-density lipoprotein-cholesterol (HDL-C) levels.[1] In general abnormality of at least one component of serum lipids, that is, high TC, high LDL-C, low HDL-C and high TG represents dyslipidemia.[2] It is one of the major contributors to the development of various diseases like atherosclerosis, cardiovascular disease (CVD), and stroke.[3] Therefore, the timely diagnosis, proper treatment and management of dyslipidemia is crucial.[4] Treatment mostly includes the use of statin with LDL-C as a major target.[5] Along with drug, lifestyle changes like increasing physical activity, changing the diet plan, and reducing total calorie intake are intended to reduce morbidity and mortality that are associated with dyslipidemia.[6] Among the widely used drug, statin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) inhibitor is known to be the most effective class of drugs for the treatment for lipid disorders.[7] Statins are considered to be the first line of therapeutic drugs for the treatment of hypercholesterolemia.[8] They are effective on lowering LDL-C and even lowering TG level and increasing HDL-C, thus can reduce risk of heart attack by 25 to 30%.[9,10] The most commonly used class of statins is lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, and rosuvastatin. Among several statins, atorvastatin is one of the well-established members of statin which has efficacy and safety across its dosage range.[11] In Nepal, among various statins preferred for treatment of dyslipidemia, atorvastatin is the most popular and the most commonly prescribed one even though different types of statins ought to have similar efficacy.[12]

Statins are the wonder drugs for lowering lipid but still there is wide interpatient variability in measurable response to these HMG-CoA reductase inhibitors. Individual patient genotype is found to have role in controlling responses to drug and the individual genetic polymorphism can influence response to statins.[13] Polymorphism in ABCA1 gene is associated with various lipid transport defects and wide-ranging HDL deficiency.[14] Various single nucleotide polymorphisms (SNPs) of ABCA1 are found to be associated with plasma lipid and susceptibility to coronary artery disease (CAD).[15] Human ABCA1 gene located on chromosome 9q31-q31[16] encodes transmembrane peptide, ABCA1, which is responsible for mediating transport of cholesterol, phospholipids, vitamins, and other lipophilic molecules across cell membranes. The extracellular domain of ABCA1 is responsible for binding to apoA-I.[17,18] Lipid-free apoA-I bound to extracellular domain is loaded with cellular phospholipids and cholesterol, which efflux excess cellular cholesterol from peripheral cell to form nascent HDL. In this way, ABCA1 has important role in the process of reverse transport of cholesterol from peripheral tissue to the liver through HDL. So, ABCA1 is considered to be preventive factor against atherosclerosis and CVDs.[14,19–21] Among various polymorphisms in ABCA1 gene, one of the most common missense polymorphism in its coding region is rs2230806, also known as Arg219Lys (R219K). The rs223080 (G) allele encodes the amino acid arginine (R) and (A) allele encodes lysine (K).[22]

R219K polymorphism is a SNP that occurs as a result of G to A transversion at nucleotide position 1051 in exon 7 that results in the substitutions of a lysine for arginine at amino acid residue 219 of ABCA1 protein.[23,24] And the codon 219 is located at long extracellular loop of ABCA1 and the substitution of an arginine by lysine alters the conformation of the extracellular domain of ABCA1 protein. This leads to increase in the activity of ABCA1 protein that can then increase the efficiency of intracellular lipid transport. It is more likely that R219K variant acts as a functional mutation to regulate HDL-C level. R219K polymorphism is found to alter the development and severity of CAD through alteration of lipid profile.[16] The R219K has been identified to be associated with protection against CAD risk and severity and this protective effect is supposed to be related to an increased HDL-C and/or reduced TG levels.[17] One probable mechanism how R219K polymorphism can reduce risk of coronary heart disease (CHD) is that R219K polymorphism can induce the activity of ABCA1 protein that helps in cholesterol efflux from peripheral cells back to liver and this process can occur without being dependent on plasma HDL-C levels. Several studies have shown that the R219K polymorphism is associated with the variation in lipid parameter of individuals.[18,23] However, the prevalence of genetic polymorphism remarkably differs among ethnic groups[24] and to the best of our knowledge, not a single study has been done in this gene among Nepalese population till date. Therefore, this study was designed with the aim to examine the effects of the R219Kpolymorphism of *ABCA1* on serum lipid levels and responses to atorvastatin therapy in Nepalese patients with dyslipidemia visiting Manmohan Cardiothoracic Vascular and Transplant Center (MCVTC), Kathmandu, Nepal.

Materials and Method

Subjects and Study Protocol

Patients newly diagnosed as having dyslipidemia and taking atorvastatin (10mg) as lipid lowering drugs for the last 3 months, visiting cardiology department of MCVTC, Institute of Medicine (IOM), Nepal, were enrolled in this study. Furthermore, patients providing written consent for the study were only included in the study. Serum and whole blood sample was collected from 88 individuals who fulfilled those inclusion criteria. Patients using drug other than atorvastatin as a lipid lowering agent and patients with diabetes mellitus, liver or renal failure, previous myocardial infarction and liver or kidney transplantation were excluded from the study. Written consent was also taken from every participant. The proposal of this study was approved by the Institutional Review committee (IRC), Institute of Medicine (IOM), Kathmandu, Nepal (IRC reference number: 320(6–11-E) 2/074/075).

Blood Chemistry Analysis

Serum total cholesterol, TG, HDL-C, and LDL-C cholesterol were estimated by enzymatic method with standard protocol used daily in clinical biochemistry laboratory of the IOM. The test was performed by commercially available enzyme assay kits by *Randox, UK*, in the fully automated chemistry analyzer, Dimension RxL Max, Siemens, Germany. Very low-density lipoprotein (VLDL) was calculated by dividing TG value (in mg/dL) by 5. TG/HDL ratio and TC/HDL ratio were also calculated.

DNA Extraction and Genotyping of the R219K Polymorphism of the ABCA1 Gene

Genomic DNA was first extracted from whole blood sample by a manual method using the protocol by Iranpur et al.[25] Isolated DNA was then amplified by polymerase chain reaction (PCR) technique using cleaver scientific-thermal and gradient thermal cycler. In PCR, a 309 bp fragment of ABCA1 gene was amplified using specific primers. The forward primer sequence: 5′-AAAGACTTCAAGGACCCAGCTT-3′ and reverse primer sequence: 5′-CCTCACATTCCGAAAGCATTA-3′ was selected for amplification based on previously described PCR assay for R219K genotyping.[26] The amplified gene was then cleaved by restriction endonuclease *EcoNI*, producing fragments of 309 bp for R allele and fragments of 184bp and 125bp for K allele (►**Fig. 1**). The fragments obtained after restriction digestion were analyzed in the 3% agarose gel electrophoresis and the result was visualized under ultraviolet transilluminator. Wild-type alleles RR showed a single band of 309 bp (no digestion). The KK homozygotes variant genotype showed two bands of 184 bp and 125 bp fragments when digested with *EcoN1*. However, for heterozygotes *R219K* polymorphic alleles (R/K), three bands of 309bp, 184bp, and 125 bp were observed.

Data Analysis

All the data were entered on Microsoft excel program. The statistical analysis was then performed by using SPSS, version 20.0. The data were presented as mean ± standard deviation (SD). To compare the lipid parameter before and after use of atorvastatin for 3 months, paired student's *t*-test was used. For the comparison of the differences in the degree of reduction of plasma lipid concentration between two ABCA1 genotype groups, one-way analysis of variance and unpaired student's *t*-test was used. The genotypic frequency was calculated by Hardy-Weinberg calculator and the deviation of the allelic and genotypic distributions from the Hardy-Weinberg equilibrium was assessed using chi-square

Fig. 1 Restriction fragment length polymorphism fragments of different samples. Lane 1 shows the DNA ladder; Lane 2, 4, 5, and 7 are undigested polymerase chain reaction product representing wild-type homozygote RR genotype; lane 8 represents homozygote KK and lane 3 and 6 represent heterozygotes RK.

Table 1 Baseline parameters of participants in different genotypes

Parameters	RR ($n = 37$)	RK ($n = 43$)	KK ($n = 8$)	p-Value
	Mean ± SD	Mean ± SD	Mean ± SD	
Age (years)	54.56 ± 12.26	58.02 ± 10.06	52.25 ± 9.19	0.227
Height (m)	1.577 ± 0.08	1.58 ± 0.07	1.57 ± 0.08	0.743
Weight (kg)	67.63 ± 11.92	63.37 ± 9.81	65.37 ± 4.13	0.198
BMI (kg/m^2)	27.12 ± 3.88	25.05 ± 3.13	26.71 ± 3.39	0.031[a]
Waist (cm)	94.63 ± 9.55	90.93 ± 11.56	95.37 ± 6.85	0.227
Hip (cm)	100.35 ± 6.20	97.36 ± 7.93	101.37 ± 7.55	0.118
W:H Ratio	0.94 ± 0.06	0.93 ± 0.07	0.94 ± 0.06	0.809
TG_0 (mg/dL)	219.83 ± 127.22	209.98 ± 150.164	347.37 ± 250.54	0.066
TC_0 (mg/dL)	193.85 ± 49.18	183.85 ± 48.3543	189.50 ± 28.67	0.643
LDL-C_0 (mg/dL)	117.97 ± 40.25	114.29 ± 43.365	96.85 ± 27.91	0.463
HDL-C_0 (mg/dL)	35.56 ± 10.20	35.92 ± 12.485	39.75 ± 14.54	0.654
VLDL_0 (mg/dL)	43.96 ± 25.44	41.99 ± 30.03	69.47 ± 50.10	0.066
TG/HDL ratio _0	6.56 ± 3.42	6.196 ± 3.79	10.53 ± 10.36	0.051
TC/HDL ratio_0	5.69 ± 1.65	5.428 ± 1.59	5.11 ± 1.36	0.584
TG_3 (mg/dL)	162.62 ± 96.38	137.25 ± 75.29	164.12 ± 54.50	0.359
TC_3 (mg/dL)	132.45 ± 36.07	127.69 ± 31.66	128.75 ± 31.55	0.816
HDL-C_3 (mg/dL)	37.89 ± 13.41	42.32 ± 18.75	43.00 ± 20.08	0.460
LDL-C_3 (mg/dL)	79.86 ± 31.87	73.49 ± 26.93	69.70 ± 34.41	0.528
VLDL_3 (mg/dL)	32.52 ± 19.27	27.45 ± 15.05	32.82 ± 10.90	0.539
TG/HDL ratio _3	4.86 ± 3.09	3.56 ± 2.20	3.56 ± 2.20	0.083
TC/HDL ratio_3	3.74 ± 1.14	3.26 ± 1.02	4.14 ± 4.07	0.215

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; SD, standard deviation; TG, triglyceride; TC, total cholesterol; VLDL-C, very low-density lipoprotein-cholesterol; WHR, waist: hip ratio; 0 = value before medication; 3 = value at 3 months.
Values are the mean ± SD.
[a]Statistically significant at $p < 0.05$; one-way ANOVA.

(χ^2) test. Two-tailed p-value less than 0.05 (with 95% confidence interval) was considered statistically significant.

Results

Baseline Parameters among Genotypes

The baseline demographic characteristics and clinical variables of the studied population are summarized in ►**Table 1**. There was no significant difference in any one of the measured parameters among the R219K variant except body mass index in studied group.

Genotypic Frequency and Distribution

The frequency of R allele of R219K polymorphism was found to be 0.665 and that of K allele to be 0.335. The genotype frequency of the R219K polymorphism of ABCA1 gene in dyslipidemia patients for genotypes RR (homozygote), RK (heterozygote), and KK (homozygote) were 42.0, 48.9, and 9.1% respectively. The genotype frequency was in Hardy-Weinberg equilibrium with $\chi 2 = 0.82$ and p more than 0.05. The genotype and allele frequencies of R219K polymorphism of ABCA1 gene are shown in ►**Table 2**.

Table 2 Frequencies of the genotype and allele of ABCA1 R219K polymorphism in patients with dyslipidemia

		Genotypes frequency			Allele frequency		
Group	n	RR (%)	RK (%)	KK (%)	R allele	K allele	χ^2
Total	88	37 (42.0%)	43 (48.9%)	8 (9.1%)	0.664	0.335	0.82
Male	53	23 (43.4%)	26 (49.1%)	4 (7.5%)	0.679	0.320	0.84
Female	35	14 (40.0%)	17 (48.6%)	4(11.4%)	0.642	0.357	0.12

Hardy–Weinberg equilibrium; R219K: $\chi 2 = 0.82$ ($p > 0.05$).
Calculated using the online genetic statistical software package, SNPstats.

Table 3 Serum lipid concentration before and after atorvastatin use

Parameters	Before medication	After medication	*p*-Value
TG (mg/dL)	226.617 ± 155.341	150.361 ± 83.585	<0.001[a]
TC (mg/dL)	188.576 ± 47.104	129.792 ± 33.286	<0.001[a]
LDL-C (mg/dL)	114.456 ± 41.009	75.262 ± 29.339	<0.001[a]
HDL-C (mg/dL)	36.123 ± 11.693	40.526 ± 16.788	0.014[b]
VLDL-C (mg/dL)	45.323 ± 31.068	30.072 ± 16.717	<0.001[a]
TG/HDL ratio	6.746 ± 4.683	4.244 ± 2.819	<0.001[a]
TC/HDL ratio	5.511 ± 1.597	3.550 ± 1.571	<0.001[a]

Abbreviations: HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride; VLDL-C, very low-density lipoprotein-cholesterol.
[a]Statistically significant at $p < 0.01$; paired sample *t*-test.
[b]Statistically significant at $p < 0.05$; paired sample *t*-test.

Effect of Atorvastatin in Serum Lipid Levels

The comparison of lipid profile value in patients before and after use of atorvastatin shows that the value of TG, TC, VLDL-C, LDL-C, TG/HDL-C ratio, and TC/HDL-C ratio reduced significantly ($p < 0.01$) and HDL-C increased significantly ($p < 0.05$) following atorvastatin treatment. The mean ± SD value of lipid parameters before and after treatment with atorvastatin for 3 months along with *p*-value is shown in ►**Table 3**.

Effect of the R219K Polymorphism of ABCA1 Gene on Serum Lipid Levels following Atorvastatin Treatment

Evaluation of mean percentage change of lipid parameter among three different genotypes (RR, RK, and KK) revealed that mean percentage decrease of TG/HDL-C was significantly different among these three genotypes with *p*-value of 0.045 and mean percentage decrease of TC/HDL-C ratio was also significantly different among three different genotypes with *p*-value of 0.047. However, there was no significant difference in percentage change in value of TG, TC, LDL-C, VLDL-C, and HDL-C among different genotypes. The association of R219k polymorphism and mean percentage change, that is, increase in HDL-C and decrease in other lipid parameters, is shown in ►**Table 4**.

Pair Comparison Showing Effect of R219K on the Effectiveness of Atorvastatin Treatment

Percentage change of lipid parameters was analyzed in pair comparisons between wild genotype RR versus variant genotype RK and KK which showed that the TG, VLDL, TG/HDL

Table 4 Mean and pair comparison percentage change in lipid profile among different genotypes of ABCA1 R219K polymorphism

Mean % change in lipid profile	RR	RK		KK	*p*-Value
TG % change	24.23 ± 18.10	29.44 ± 20.93		42.19 ± 23.55	0.070
TC % change	29.79 ± 17.14	28.25 ± 16.36		32.68 ± 10.65	0.758
LDL% change	28.44 ± 26.07	31.29 ± 22.80		37.58 ± 16.06	0.628
HDL % change	2.02 ± 22.19	10.48 ± 22.75		12.05 ± 8.978	0.190
VLDL % change	24.23 ± 18.18	29.44 ± 20.93		42.19 ± 23.55	0.070
TG/HDL ratio %change	25.88 ± 23.27	37.89 ± 28.59		45.98 ± 16.98	0.045[a]
TC/HDL ratio %change	32.14 ± 18.34	40.89 ± 15.51		42.93 ± 10.31	0.047[a]
Pair comparison of % change in lipid profile	**RR**	**RK**	***p*-Value**	**KK**	***p*-Value**
TG	24.23 ± 18.18	29.44 ± 20.93	0.242	42.19 ± 23.55	0.021[a]
TC	29.79 ± 17.14	28.25 ± 16.36	0.681	32.68 ± 10.65	0.652
LDL-C	28.44 ± 26.07	31.29 ± 22.80	0.604	37.58 ± 16.06	0.378
VLDL	24.23 ± 18.18	29.44 ± 20.93	0.242	42.19 ± 23.55	0.021[a]
HDL-C	2.022 ± 22.19	10.48 ± 22.75	0.101	12.05 ± 8.978	0.050
TG/HDL ratio	25.88 ± 23.27	37.89 ± 28.59	0.046[a]	45.98 ± 16.98	0.026[a]
TC/HDL ratio	32.145 ± 18.347	40.892 ± 15.518	0.025[a]	42.930 ± 10.317	0.046[a]

Abbreviations: ANOVA, analysis of variance; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TG, triglyceride; TC, total cholesterol; VLDL-C, very low-density lipoprotein-cholesterol.
[a]Statistically significant at $p < 0.05$; one-way ANOVA and unpaired sample *t*-test.

ratio, TC/HDL ratio decreases significantly with higher percentage in KK genotypes than in RR genotype ($p < 0.05$). While these parameters when compared between RR and RK genotypes, only TG/HDL ratio and TC/HDL ratio were found significantly decreased with greater percentage in RK genotype than RR genotype ($p < 0.05$). But there was no significant percentage reduction in value of TC and LDL-C with RK and KK genotypes than RR genotypes. The HDL-C was increased with higher percentage in KK genotype than in RR with $p = 0.05$ (►**Table 4**). These results indicated that polymorphic genotype either RK or KK genotype is associated with greater degree of improvement in lipid level than nonpolymorphic RR genotype. These results also indicated that KK genotype of R219K polymorphism has association with greater degree of improvement of lipid profile values following atorvastatin treatment.

Discussion

Dyslipidemia is a condition with derangement of lipid and/or lipoproteins level including increased total cholesterol level or TGs level or LDL cholesterol level or decreased level of HDL cholesterol level. It is considered as a major risk factor for CVDs.[27] Therefore, the prevention and sensible management of dyslipidemia can greatly alter the CVDs risk and related morbidity and mortality.[28] Dyslipidemia is treated with numbers of medication, the most common being statins.[29] The action of the statin in both lipid modifying and reducing risk of CVDs is found to be variable in various patient population receiving similar drug as same dose. The heterogeneity in lowering TC, LDL-C, TG, and increasing HDL-C was found in various patients' population even at the highest statin doses available. The cause behind this may be various polymorphisms in genes that are responsible for modulating the action of statins. Variability in gene responsible for statin absorption, distribution, and/or excretion may also affect several drug-related phenotypes.[30] Various SNPs are associated with drug metabolism and their actions like SNPs in ABCA1 gene may play role in the different responses to the lipid lowering therapy.[17] One of the frequent R219K SNP of ABCA1 genes is found to be associated with decreased severity of atherosclerosis, decreased TG levels, and increased HDL-C levels. Consequently, R219K seems to be associated with decreased risk and severity of CAD.[18] But the results obtained from different studies are not replicable.[17] Also, similar studies are not performed in Nepalese population yet.

The result of this study revealed that there was significant association of R219K polymorphism with higher degree of percentage reduction in value of TG/HDL ratio, and TC/HDL ratio in atorvastatin users. However, no association of R219K polymorphism was seen in reduction of serum TC, LDL-C, TG, and VLDL levels and increase of HDL-C levels. The effect of R219K seen in TG, VLDL, TG/HDL, and HDL in this study is also reported earlier.[17,18,31,32] Contrast to this, in a study by Kolovou et al,[26] no association was observed between R219K gene polymorphism and demographic and lipid parameters. In addition to our study, Akao et al[33] also demonstrated nonsignificant relationship between the presence and absence of R219K variant and statin-induced LDL-C response or changes in other lipid profiles including TG or HDL-C levels.

The allele frequency (R allele = 0.665 and K allele = 0.335) and genotype frequency (RR = 42.0%, RK = 48.9%, and KK= 9.1%) of R219K polymorphism found in this study is almost similar to allele frequency (R allele = 0.617 and K allele = 0.383) and genotype frequency (RR = 38.3%, RK = 46.7%, and KK= 16.1%) reported by Li et al. in Chinese population.[34] The genotype frequency (RR = 50.5%, RK = 42.8%, and KK= 6.7%) published by Kolovou et al,[26] in Greek population also showed similar frequency with our finding, major similarity being in the KK genotype (6.7%).

Our study also reports that mean percentage reduction in TG/HDL-C ratio, TC/HDL ratio, and TG and VLDL was significantly higher in individuals having KK genotypes than those having RR genotypes ($p < 0.05$). Consistent with our results, Genvigir et al[17] demonstrated that the carriers of 219 RK/KK genotypes had lower TG/HDL-C ratio in comparison to that of 219 RR genotype and 219 RK/KK carrier had lower basal serum TG and VLDL than those with 219 RR carrier. Wu et al[23] also demonstrated that subjects carrying K allele had significantly decreased TC/HDL-C ratio when compared with RR genotype. Therefore, our study could provide the supporting fact that rare allele of R219K SNP showed association with decrease in TG/HDL-C and TC/HDL-C ratio. The study done by Lutucuta et al[18] and Clee et al[31] also found that R219K variant is associated with decrease in TG level. Further, our study also demonstrated that the mean percentage increase in HDL-C was higher in KK genotype than in RR genotype ($p = 0.05$). This favors the fact that homozygous K genotype is associated with higher rate of rise in HDL-C as compared with other in statin users. Similar to our study, the study conducted in Chinese population also demonstrated that the action of statin on HDL-C levels is modified by R219K polymorphism of ABCA1 gene and they also found that the mean percentage rise in HDL-C was higher in patients who were homozygous for KK than that in patients who were homozygous for RR. This supports the finding of our study that HDL-C increasing effect of statin can be affected by R219K polymorphism.[34]

Our study is also in accordance with meta-analysis of Mokuno et al[35] which analyzed that the HDL-C concentration was higher in both men and women with RK or KK genotype than those with RR genotype. Moreover, the study conducted by Maruyama et al[36] supports the evidence that serum HDL-C level of homozygotes of K219 allele is higher than those of carriers of homozygous R219 allele. All these findings suggest that 219KK allele is an antiatherogenic allele that might contribute in cholesterol efflux activity. Moreover, the degree of percentage reduction in TG/HDL-C ratio and TC/HDL ratio was significantly higher in individuals with RK genotypes than in individual with RR genotype ($p < 0.05$). This indicates that carrier of K allele of R219K polymorphism either of heterozygous or homozygous types is associated with greater reduction in TC/HDL-C ratio and TG/HDL-C in comparison to nonpolymorphic RR genotype. Thus, it can be

concluded that the lipid rising or lipid reduction response of atorvastatin could be affected by R219K polymorphism of ABCA1 gene mainly by K allele of R219K.

However, this study could not provide the evidence for mechanism by which R219K polymorphism affects the statin effect in improving lipid parameters. Also, best to our knowledge there are no studies that have assessed the mechanism by which R219K ABCA1 genetic variant affects responses to statin treatment in terms of lipid modification.

Our observations could provide a possible genetic explanation for this finding, but our study was conducted in a sample of modest size and also this study does not determine the effects of other polymorphism of ABCA1 gene that are associated with lipid level and lipid lowering action of statin. This study also could not provide the possible explanation for observations. Thus, further clinical studies need to be conducted in large sample size including effect of other genetic variants to extend our observation to wide range of population.

Conclusion

This is the first study conducted in Nepal to demonstrate the frequency of *ABCA1* R219K polymorphism and its effect in the lipid parameters and the lipid lowering action of statins. Our study concluded that among the statin users, the KK genotype of R219K polymorphism is associated with greater degree of reduction in TG, VLDL, TG/HDL ratio and TC/HDL ratio. It was also demonstrated that carrier of K allele relative to carrier of R allele had significantly greater reduction in value of TG, VLDL, TG/HDL ratio and TC/HDL ratio and higher level of increase in level of HDL-C. This result might indicate the K allele to be protective against subclinical cardiovascular disease in Nepalese population. In conclusion, R219K polymorphism of ABCA1 gene might be a novel genetic determinant of statin treatment response in patients with dyslipidemia.

Data Availability

All the data used and/or analyzed in this study can make available from the corresponding author on request.

Patient Consent

The informed consent was obtained from all the individual patients.

Ethical Approval

This study was approved by the Institutional Review committee (IRC), Institute of Medicine (IOM), Kathmandu, Nepal (IRC reference number: 320(6–11-E) 2/074/075).

Consent for Publication

I, Binod Kumar Yadav, corresponding author of this manuscript give full consent for publication on behalf of all co-authors.

Authors' Contribution

S.G. performed laboratory work, statistical analysis, and manuscript writing. BKY helped in designing the study, analysis of the data, and writing of the manuscript and proof reading. J.S. contributed to laboratory work standardization, data analysis, and proof reading. S.S. and D.S. supported in laboratory work. V.K.S., E.T.T., M.R., and A.B. conceived the study and participated in the design and coordination of the study. K.D.M., C.M.P., and V.P. read and approved the final manuscript.

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Conflicts of Interest

None declared.

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References

1 Kang YJ, Wang HW, Cheon SY, Lee HJ, Hwang KM, Yoon HS. Associations of obesity and dyslipidemia with intake of sodium, fat, and sugar among Koreans: a qualitative systematic review. Clin Nutr Res 2016;5(04):290–304
2 Lin C-F, Chang Y-H, Chien S-C, Lin Y-H. Yeh H-Y. Epidemiology of dyslipidemia in the Asia Pacific region. Int J Gerontol 2018;12 (01):2–6
3 Qi L, Ding X, Tang W, et al. Prevalence and risk factors associated with dyslipidemia in Chongqing, China. Int J Environ Res Public Health 2015;12(10):13455–13465
4 Sukhorukov V, Karagodin V, Orekhov AN. Modern methods of diagnosis dyslipidemia. Patol Fiziol Eksp Ter 2016;60(01): 65–72
5 Parhofer KJDI. Update dyslipidemia. 2013;54(9):1089–103
6 Jacobson TA, Ito MK, Maki KC, et al. National lipid association recommendations for patient-centered management of dyslipidemia: part 1—full report. J Clin Lipidol 2015;9(02):129–169
7 Bonfim MR, Oliveira ASB, Amaral SLd, Monteiro HL. Treatment of dyslipidemia with statins and physical exercises: recent findings of skeletal muscle responses. Arq Bras Cardiol 2015;104: 324–331
8 Clark LT. Treating dyslipidemia with statins: the risk-benefit profile. Am Heart J 2003;145(03):387–396
9 Ramkumar S, Raghunath A, Raghunath S. Statin therapy: review of safety and potential side effects. Acta Cardiol Sin 2016;32(06):631
10 Mandal CC. High cholesterol deteriorates bone health: new insights into molecular mechanisms. Front Endocrinol (Lausanne) 2015;6:165
11 Jones PH, McKenney JM, Karalis DG, Downey J, Journal NIJAH. Comparison of the efficacy and safety of atorvastatin initiated at different starting doses in patients with dyslipidemia. Am Heart J 2005;149(01):e1–e8
12 Bhattarai AK, Acharya A, Karki PK. Use of statins as lipid lowering agent in hypercholesterolemia in a tertiary care hospital: a descriptive cross-sectional study. J Nepal Med Assoc 2020;58 (232):1031–1035

13 Kansu B, Lang D. Genetic polymorphisms as predictive markers for statin therapy: a route to improved cardiovascular patient outcomes? JBHTIJoSR 2017;10
14 Ergen A, Isbir S, Tekeli A, Isbir T. Investigation of ABCA1 C69T and G-191C polymorphisms in coronary artery disease. In Vivo 2008; 22(02):187–90
15 Guo Z, Wu P, Xie D, Wang Q, Liu Y, Cha Z, et al. A new discovered ABCA1 gene polymorphisms and the association of ABCA1 SNPs with coronary artery disease and plasma lipids in Chinese population. 2011;26(4):179–90
16 Ghaznavi H, Aali E, Soltanpour MS. Association study of the ATP-binding cassette transporter A1 (ABCA1) Rs2230806 genetic variation with lipid profile and coronary artery disease risk in an Iranian population. Open Access Maced J Med Sci 2018;6(02): 274–279
17 Genvigir FDV, Soares SA, Hirata MH, et al. Effects of ABCA1 SNPs, including the C-105T novel variant, on serum lipids of Brazilian individuals. Chin Chim Acta 2008;389(1–2):79–86
18 Lutucuta S, Ballantyne CM, Elghannam H, Gotto AM Jr, Marian AJ. Novel polymorphisms in promoter region of ATP binding cassette transporter gene and plasma lipids, severity, progression, and regression of coronary atherosclerosis and response to therapy. Circ Res 2001;88(09):969–73
19 Ceccanti M, Cambieri C, Frasca V, Onesti E, Biasiotta A, Giordano C, et al. A novel mutation in ABCA1 gene causing Tangier disease in an Italian family with uncommon neurological presentation. Front Neurol 2016;7:185
20 Saleheen D, Khanum S, Haider SR, Nazir A, Ahmad U, Khalid H, et al. A novel haplotype in ABCA1 gene effects plasma HDL-C concentration. Int J Cardiol 2007;115(01):7–13
21 Wang N, Silver DL, Thiele C, Tall AR. ATP-binding cassette transporter A1 (ABCA1) functions as a cholesterol efflux regulatory protein. J Biol Chem 2001;276(26):23742–7
22 Zargar S, Wakil S, Mobeirek AF, Al-Jafari AA. Involvement of ATP-binding cassette, subfamily A polymorphism with susceptibility to coronary artery disease. Biomed Rep 2013;1(06):883–8
23 Wu Y, Bai H, Liu R, Liu Y, Liu B. Analysis of ATP binding cassette A1 gene R219K polymorphism in patients with endogenous hypertriglyceridemia in Chinese population. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2007;24(02):177–181
24 Pontoriero AC, Trinks J, Hulaniuk ML, Caputo M, Fortuny L, Pratx LB, et al. Influence of ethnicity on the distribution of genetic polymorphisms associated with risk of chronic liver disease in South American populations. 2015;16(1):1–8
25 May S Rapid Extraction of High Quality DNA from Whole Blood Stored at 4°C for Long Period
26 Kolovou V, Marvaki A, Boutsikou M, Vasilopoulos G, Degiannis D, Marvaki C, et al. Effect of ATP-binding cassette transporter A1 (ABCA1) gene polymorphisms on plasma lipid variables and common demographic parameters in Greek nurses. 2016;10:233
27 Pradeepa R, Mohan V. Epidemiology of Dyslipidemia in India. 2017
28 Kopin L. Lowenstein CJJAoim. Dyslipidemia. 2017;167(11): ITC81–ITC96
29 Sorace P, LaFontaine T, Thomas TR. Know the risks: lifestyle management of dyslipidemia. ACSM's Health Fit J 2006;10(04): 18–25
30 Zineh I. Pharmacogenetics of response to statins. Curr Atheroscler Rep 2007;9(03):187–194
31 Clee SM, Zwinderman AH, Engert JC, et al. Common genetic variation in ABCA1 is associated with altered lipoprotein levels and a modified risk for coronary artery disease. Circulation 2001; 103(09):1198–1205
32 Mantaring M, Rhyne J, Hong SH, Miller M. Genotypic variation in ATP-binding cassette transporter-1 (ABCA1) as contributors to the high and low high-density lipoprotein-cholesterol (HDL-C) phenotype. Transl Res 2007;149(04):205–210
33 Akao H, Polisecki E, Schaefer EJ, et al. ABCA1 gene variation and heart disease risk reduction in the elderly during pravastatin treatment. Atherosclerosis 2014;235(01):176–181
34 Li J, Wang LF, Li ZQ, Pan W. Effect of R219K polymorphism of the ABCA1 gene on the lipid-lowering effect of pravastatin in Chinese patients with coronary heart disease. Clin Exp Pharmacol Physiol 2009;36(5-6):567–570
35 Mokuno J, Hishida A, Morita E, et al. ATP-binding cassette transporter A1 (ABCA1) R219K (G1051A, rs2230806) polymorphism and serum high-density lipoprotein cholesterol levels in a large Japanese population: cross-sectional data from the Daiko Study. Endocr J 2015;•••:EJ14–EJ0577
36 Maruyama C, Imamura K, Teramoto T. Assessment of LDL particle size by triglyceride/HDL-cholesterol ratio in non-diabetic, healthy subjects without prominent hyperlipidemia. J Atheroscler Thromb 2003;10(03):186–191

