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Original Research Article

EVALUATING THE ASSOCIATION BETWEEN APOLIPOPROTEIN A5 SINGLE NUCLEOTIDE POLYMORPHISMS (APOA5 SNPS) AND HYPERTRIGLYCERIDEMIA

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Abstract: *The retrospective study was conducted at the Faisalabad Institute of Cardiology from January 2022 to January 2023 to evaluate the association between apolipoprotein A5 single nucleotide polymorphisms (APOA5 SNPS) and hypertriglyceridemia in the Pakistani population and assess the efficacy of fibrate therapy in hypertriglyceridemic patients. Blood samples of patients were collected for DNA extraction by the standard inorganic method. Extracted DNA was analyzed biochemically and genetically following the selection and genotyping of the APOA5 gene to know SNP variants with risk alleles. We analyzed lipid profiles, including TG (triglyceride), HDL (high-density lipoprotein), and LDL (low-density lipoprotein) levels of hypertriglyceridemic patients, then compared them to normal and fibrate-treated individuals. We compared APOA5 (rs662799) SNP (single nucleotide polymorphism) among 50 hypertriglyceridemic, 50 healthy controls, and among the same 50 hypertriglyceridemic patients who were given fibrate. Results showed that fibrate decreased TG level and LDL by about 30-45% and 20-25%, respectively; increased high-density lipoprotein by 10-15% and total cholesterol decreased by 6-8% in Pakistani. Hypertriglyceridemia risk was significantly increased by a minor allele of APOA5 rs662799 polymorphism. Minor allele carriers of rs662799 had an odd ratio of (95% CI) 1.5 (1.03-2.18) (P = 0.032). Risk allele frequency differed among hypertriglyceridemic patients, healthy controls, and fibrate-treated hypertriglyceridemic individuals. We analyzed that 87.5% of healthy controls exhibited no risk allele, while 78% did not show this risk allele among hypertriglyceridemic patients. It was concluded that APOA5 rs662799 polymorphism is a genetic determinant of hypertriglyceridemia. Fibrate caused a reduction in TG and LDL and caused an elevation of HDL-C among hypertriglyceridemic patients.*

Keywords: Hypertriglyceridemia, Single Nucleotide Polymorphism, Fibrate therapy

Introduction

Hypertriglyceridemia is a feature of various metabolic disorders, including dyslipidemia, metabolic syndrome, and type 2 diabetes mellitus. This disorder can lead to an enhanced risk of premature coronary artery. The prevalence of hypertriglyceridemia has been rapidly increasing Ruiz-García et al., 2020). Triglycerides are composed of chylomicrons and very low-density lipoproteins. The liver secretes triglycerides in the form of very low-density lipoprotein during fasting conditions and after a meal (Yu et al., 2023). Apolipoproteins A5 (APOA5) are lipid-binding proteins found on the surface of plasma lipoprotein particles that act as structural components and ligands for various proteins and receptors. Among humans, the chromosomal location of the APOA5 gene is 11q23, which contains three introns and four exons. APOA5 gene was recognized as a sequence of DNA with the size of 27 kb is converted and located proximally to the APOA1/C3/A4 gene cluster. Human APOA5 is solely expressed in the liver leading to exportation into plasma being associated with particles of high-density lipoprotein (HDL) and very low-density lipoproteins (VLDL) (Chen et al., 2021). Humans contain only 0.1-0.4 g/ml of this plasma protein, which means that only one of the APOA5 molecules is found among 24 particles of VLDL.

It has been found that APOA5 strongly modulates lipids in the blood. Triglyceride level is lowered when APOA5 is increased in plasma. It prevents the production of very low-density lipoprotein triglyceride and triggers the hydrolysis of very low-density lipoprotein triglyceride. It has an important

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role in modulating the metabolism of other blood lipids (Park and Kang, 2020). Single nucleotide polymorphisms (SNPs) are commonly found in humans. Numerous SNPs, mainly rs662799 located on the promoter region of the APOA gene, are associated with raised levels of triglyceride (Hechmi et al., 2020; Vrablik et al., in this study, we will evaluate the association between APOA5 SNPs and hypertriglyceridemic in Pakistani population, and assess the efficacy of fibrate therapy in hypertriglyceridemic patients.

Methodology

The retrospective study was conducted at the Faisalabad Institute of Cardiology from January 2022 to January 2023. Both males and females aged ≥20 years were included in the study. The included individuals took fibrate 2 weeks before or after a cardiac attack. Lipidemic patients and pregnant women were excluded. A total of 100 individuals who fulfilled the inclusion criteria were included in the study. Informed consent of the participants was taken. The ethical board of the hospital approved the study. Study participants were divided into three groups: Group I (n=50) contained healthy controls, and Group II (n=50) contained hypertriglyceridemic patients. Blood samples were collected in EDTA blood vials to ensure anticoagulation. Collected blood samples were put into an icebox; after reaching the research lab, samples were preserved at -20^{o}C in a freezer until the DNA extraction. Human genomic DNA was extracted by an inorganic method using NaCl from 3ml of blood samples (Dairawan and Shetty, 2020). The nanodrop procedure was then utilized for measuring DNA purification.

Single nucleotide polymorphism (SNP) at the APOA5 gene promoter site was detected using polymerase chain reaction (PCR). High-resolution Melt (HRM) was used for genotyping SNP of APOA5. Features of selected SNP are summarized in Table I. Sequence of selected SNP of APOA5 was attained from NCBI (National Centre for Biotechnology Information). Only exon sequence was used for primer designing to prevent genomic DNA contamination. Table II shows the primer's annealing temperature, amplicons size, and SNP to be analyzed. Primers were optimized through PCR, and PCR products were then separated, based on their size, by gel electrophoresis. Two specific primer sets were designed for determining SNP genotypes. Fragments containing SNP were amplified using real-time PCR, and variations in DNA sequence were identified by high-resolution melting curve analysis (HRMCA), which monitors changes in the melting of DNA duplex.

Data were analyzed using SPSS version 23.0. The standard for confirming SNP was when its frequency was higher than 0.1. The chi-square test was used to evaluate the difference in SNP frequency among all study groups. Risk alleles were assessed using an odd ratio. P value < 0.05 was considered statistically significant.

Table I Features of selected SNP

Gene	Full Name	Chromosome position	Number of amino acids	Weight (kDa)
APOA5	Apolipoprotein AV Precursor	11q23.3	343	120kD

Table II Sequence of primers with GC content and annealing temperature

Gene	Primer	GC (%)	Annealing temperature
APOA5	F:5'-CTCAGCCAGCATTCATAG-3' R:5'-AGTACTGTAGACGGAGTG-3'	50	57.6

Results

The baseline characteristics of the population are summarized in Table III**.** Lipid profiles involving TG (triglyceride), HDL (High-density lipid), and LDL (low-density lipid), along with the body mass index of HTG patients, are analyzed and then compared to normal and fibrate-treated individuals. Fibrate was given to the same HTG patients after 24 hours of observation of the lipid profile of these patients. Fibrate decreased TG levels and LDL by about 35-50% and 10-20%, increased HDL by about 10-15%, and decreased total cholesterol by 15-30% in our study population. Body mass index was an independent trait in fibrate therapy.

APOA5 rs662799 polymorphism in hypertriglyceridemic and Control groups was analyzed (Table IV). For the rs662799 genotype, the wild-type TT was found in 10(50.75%) of 50 hypertriglyceridemia cases and 28 (64%) of 50 healthy controls. Heterozygous (TC) genotype variants were found in 23(42.75%) of 50 hypertriglyceridemic cases and in 21(31%) of 50 healthy subjects (P=0.036). Homozygous CC (mutant genotype) was observed in 26(6.5%) of 50 HTG

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cases, in only 3(5%) of 50 individuals had this genotype (P= 0.0260). So, the proportion of HTG cases with the rs662799 "TT, TC and CC genotypes" was 50.75%, 42.75%, and 6.5% correspondingly; the fraction of healthy Control with the rs662799 TT, TC, and CC genotypes was 64%, 31%, and 5% respectively. The heterozygous genotype of rs662799 was found to be significantly correlated with HTG and healthy Control, as $X^2 = 4.587$, probability (95% CI) = 1.66 (1.09-2.65) (P = 0.036). Moreover, the C and T alleles correlate with healthy Control and HTG cases (p = 0.032). The ancestral allele 'T' frequency was 42(78%) in healthy Control and 47(87.5%) in HTG patients. While the frequency of risk allele 'C' was found in 18(22%) of 50 HTG patients and 10 (12.5%) of 50 healthy. The above results indicate a higher proportion of heterozygous genotype TC in HTG patients is 42.75% higher than in healthy subjects. However, a comparatively high proportion of the TT genotype was observed in healthy subjects. Since risk allele 'C' is more common among HTG patients.

A comparison of APOA5 rs662799 polymorphism before and after fibrate therapy is summarized in Table V

Table III Baseline Characteristics of the Study Population

Baseline Characteristics of the Study Population			
Characteristics	**Normal**	**Hypertriglyceridemia**	**Fibrate***
Number of Subjects	50	50	50
Age, Year	41±6.6	42±9	42±9
Gender			
Male	32 (63%)	39 (77)	39 (77)
Female	20 (38%)	11(23%)	11(23%)
Laboratory Parameters			
BMI, Kg/m^2	23.40±1.09	26.4±2.93	26.1±2.85
Serum Triglyceride, mg/dl	109.2±20.76	357.20±107.88	187.12±97.18
HDL-C, mg/dl	46.5±6.10	39.12±11.1	52.10±11.02
LDL-C, mg/dl	78.24±9.12	112.02±42.01	89.22±34.06
Serum Cholesterol, mg/dl	146.2±12.10	227.4±54.60	180.1±38.14
**** Fibrate was given to the same HTG patients after 24 hours of observation of the lipid profile of these patients.***			

Table IV Comparison of genotype rs66279 among Hypertriglyceridemic and Control groups

Gene	**Genotype**	**HTG (%) n=50**	**Control (%) n=50**	**X^2**	**Odds (95% CI)**	**P-Value**
APOA5, rs662799, C>T	**TT**	26(50.75%)	28(64%)	-	1.000(ref.)	-
	TC	23(42.75%)	21(31%)	4.587	1.66(1.09-2.65)	0.036
	CC	8.5(6.5%)	3(5%)	0.033	1.32(0.49-3.53)	0.0260
Alleles	**T (Ancestral allele)**	47(78%)	42(87.5%)	-	1.000(ref.)	-
	C (risk allele)	18(22%)	10(12.5%)	4.479	1.5(1.03-2.18)	0.032

Table V Comparison of genotype rs66279 among Hypertriglyceridemic and Fibrate treated Hypertriglyceridemic Patients

Gene	**Genotype**	**HTG (%) n=50**	**Fibrate Therapy (%) (n=Same 50 HTG)**	**X^2**	**Odds (95% CI)**	**P-Value**
APOA5, rs662799, C>T	**TT**	26 (50.75%)	28(68%)	-	**1.000(ref.)**	-
	TC	20 (42.75%)	19 (28.5%)	4.001	1.42 (1.02-2.12)	0.03
	CC	26 (6.5%)	3 (3.5%)	0.022	1.20 (0.37-3.42)	0.0201
Alleles	**T** Ancestral allele)	47 (82.5%)	42 (89.60%)	-	1.000 (ref.)	-
	C (risk allele)	18 (22%)	8.5 (10.4%)	4.479	1.56 (1.06 ~ 2.45)	0.034

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Discussion

APOA5 polymorphism has been associated with variations in plasma triglyceride levels. Different populations have varying frequencies of APOA5 SNPs (Vrablik et al., 2019). In our research study, we analyzed the lipid profile (TG, HDL, and LDL levels) of HTG patients, then compared it to normal and fibrate-treated individuals. Fibrate was given to the same HTG patients by 24-hour observation of the lipid profile of these patients. Fibrate decreased TG levels by about 30-45% and 20-25%, increased HDL by about 10-15%, and decreased total cholesterol by about 6-8% in the Pakistan population. These results in the Pakistan population were in accordance with another study which reported fibrate decreased TG level to 36.6 % and increased HDL-C by 13.7 % (Orringer et al., 2019). In another study, fibrate decreased the TG level by up to 50 %, and increased HDL-C by up to 20 %, along with a 20 % reduction in LDL-C by 20 % (de Luis et al., 2021).

The -1311T>C (rs662799) polymorphism was significantly associated with higher mean triglyceride concentrations. A significant association was found between heterozygous genotypes of HTG and Control. Similarly, a significant association was found among heterozygous genotypes of HTG and fibrate-treated HTG patients. The relation is insignificant for TT genotypes among healthy, hypertriglyceridemic, and fibrate-treated individuals. These findings are comparable with the results of the other studies conducted by Chuluun-Erdene et al. (Chuluun-Erdene et al., 2020) and Shahid et al. (Shahid et al., 2017). Single nucleotide polymorphism rs662799 frequency in our study was extremely low (P=0.04) when compared to other studies conducted on Chinese (Xie et al., 2017) and Korean populations (Kim et al., 2019).

In the current study, serum TAG concentration was higher in C/C carriers than in T/C and T/T carriers. Carriers of the C allele were at increased risk of hypertriglyceridemia compared to those with T/T genotype. Results confirmed a significant association between APOA5 polymorphism and heightened risk of HTG in the Pakistani population. Results further imply that genetic variants (such as disease predictors) may impact risk factors (Hubáček et al., 2020) and can be included in risk assessment for personalized treatment (Mirabedini et al., 2023; Xie et al., 2017). The limitation of this study is the small population size; a larger multi-centered study is recommended for further analysis.

Conclusion

It is concluded that APOA5 rs662799 polymorphism is a genetic determinant of hypertriglyceridemia. Fibrate caused a reduction in TG and LDL and caused an elevation of HDL-C among hypertriglyceridemic patients.

Conflict of interest

The authors declared the absence of conflict of interest.

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