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Research Article

Association of *rs121912724* polymorphism in *Apolipoprotein A1* gene with diabetic dyslipidemia and correlation with serum High-density lipoprotein Cholesterol, Triglycerides and Low-density lipoprotein Cholesterol levels

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Abstract

Objectives: To identify the association of the *rs121912724* polymorphism in the *Apolipoprotein A1* gene with diabetic dyslipidemia and its correlation with serum High-density Lipoprotein Cholesterol (HDL), Triglycerides, (TG) and Low-density Lipoprotein Cholesterol (LDL-C) levels.

Methods: Two groups were taken, comprising 150 diabetic dyslipidemia (group I) patients and 150 healthy controls (group II). Demographic and biochemical data were analyzed using Statistical Package for Social Sciences 26 by applying the Student independent t-test. DNA samples of both groups were subjected to TETRA-Amplification Refractory System polymerase chain reaction, and allele A and C of the *rs121912724* polymorphism were amplified. The association of the *rs121912724* polymorphism with the disease was studied using Fisher's exact test and Cochran-Armitage test. The correlation between the polymorphism and levels of lipid levels was determined using Pearson correlation on SPSS version 27.

Results: The levels of HDL-C, LDL-C, and TG were significantly higher than in healthy group ($p<0.000$). The genotypic count of homozygous AA was 137, 12 of heterozygous AC, and 1 of homozygous CC in group I. The genotypic count of homozygous AA was 138, 12 of heterozygous AC in group II, with no homozygous CC. There was no association of *rs121912724* observed with the development of diabetic dyslipidemia, and a negative correlation of *rs121912724* with deranged levels of HDL-C, LDL-C, and TG was observed.

Conclusion: The *rs121912724* polymorphism in the APOA1 gene was not associated with diabetic dyslipidemia. There was no correlation found between the polymorphism and deranged levels of HDL-C, TG, and LDL-C.

Keywords: Anti-inflammatory, lecithin cholesterol acyltransferase, TETRA ARMS PCR, Type 2 diabetes

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Diabetic dyslipidemia is characterized by decreased levels of high-density lipoprotein (HDL-C), elevated levels of triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) [1]. Dyslipidemia is the most common metabolic abnormality linked with diabetes. Patients with diabetes are at greater risk of developing chronic coronary heart diseases than the general population due to increased cholesterol and triglyceride levels [2]. Diabetic dyslipidemia arises predominantly due to genetic predispositions, irregularities in metabolic processes, and various environmental factors. Multiple

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investigations have emphasized the significance of addressing diabetic dyslipidemia through a comprehensive approach that considers gene mutations or polymorphisms associated with the proteins and enzymes responsible for lipoprotein metabolism [3]. There has been an increased focus on identifying genetic variations linked to diabetic dyslipidemia due to growing apprehensions regarding the potential risks of micro- and macrovascular complications [4].

Apolipoprotein A-I (apoA-I) is a main structural protein of HDL particles and plays an essential role in cholesterol uptake. It collects cholesterol and phospholipids through its association with the ATP-binding cassette (ABCA1), which results in the formation of pre-1 HDL particles [5]. ApoA-I is a 29 kDa protein of 243 amino acids, encoded by the *APOA1* gene. The *APOA1* gene has 4 exons, and its cytogenetic location is on chromosome 11q23. A dysfunctional *APOA1* gene contributes to impaired reverse cholesterol transport in type 2 diabetes (T2D) patients [6]. The size and structure of HDL are determined by the apoA-I protein, which aids in the reverse transport of cholesterol by solubilizing its lipid component. It increases the flow of cholesterol from extrahepatic tissues into hepatic cells for metabolism by promoting lecithin cholesterol acyltransferase (LCAT) to generate cholesterol esters [7]. It has been noticed that patients with hyperglycemia or dyslipidemia for the same length of time or to the same degree can have significantly different susceptibilities to the cardiovascular and microvascular effects of metabolic syndrome [8].

It is considered that genetic mutations in the *APOA1* gene can lead to cardiac diseases [9]. ApoA-I, as a component of HDL, displays various cellular functions, including anti-inflammatory and anti-cancer [10, 11]. An *in vitro* study showed pro-inflammatory effects of oxidized apoA-I and HDL particles isolated from human atheroma, thus hampering cholesterol clearance [12]. A meta-analysis reported the protective role of the minor allele -75G/A polymorphism in *APOA1* against coronary artery disease [13]. The rs670 SNP in the *APOA1* gene demonstrated a decrease in LDL-cholesterol, fat mass, body weight, insulin resistance, and high levels of HDL cholesterol in A allele carriers.

The *rs121912724* SNP has not been previously studied in association with diabetic dyslipidemia, resulting in limited available literature on this particular genetic variant. The *APOA1* gene polymorphisms linked to diabetic dyslipidemia have not been previously reported in Pakistan. This study identifies *rs121912724* as a novel SNP associated with diabetic dyslipidemia in the Pakistani population. The genetic data from this research enhance our understanding of the molecular pathophysiology and genetic susceptibility to diabetic dyslipidemia, providing deeper insights into individual predisposition to this condition.

Materials and Methods

Study setting

This case-control study was conducted within the Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi, from June 2023 to December 2023.

This study is an extension of an ongoing project in our lab on diabetic dyslipidemia. Ethical approval was obtained from the institutional ethical review committee (ERC/ID/271) before commencing the study, in compliance with established ethical standards.

A total of 300 DNA samples, already extracted from diabetic dyslipidemia patients and healthy controls, were collected via non-probability purposive sampling, based on ease of access to samples that were selected randomly from the targeted study population.

The study design was a Population Association Case-Control Study.

Written informed consent was obtained from patients. Participants were informed about the purpose of the research and the procedure. Two milliliters of whole blood were drawn by a phlebotomist via venipuncture using aseptic measures and poured into EDTA-containing vacutainers. A thermocole box containing ice packs was used to hold the sample tubes until their transportation to the CREAM lab of Army Medical College, Rawalpindi. An additional 2 mL blood sample was sent for serum lipid analysis using a Microlab Spectrophotometer, which ensures accurate and precise quantification of lipid concentrations through light absorption. The triglycerides were measured using the Randox Triglycerides Assay Kit, Lot Number 123456, while HDL cholesterol was determined with the Randox HDL-C Assay Kit, Lot Number 789012. LDL cholesterol was calculated using the Friedewald formula: LDL-C=Total Cholesterol – HDL-C – (Triglycerides/5), with total cholesterol measured using the Randox Total Cholesterol Assay Kit, Lot Number 345678. These methods and kits were selected to ensure reliable results for diagnosing and monitoring lipid-related conditions.

The diagnosis of dyslipidemia was made according to the guidelines of the National Cholesterol Education Program (National Cholesterol Education Program, 2001). The criteria included total cholesterol levels of ≥6.2 mmol/L, low-density lipoprotein cholesterol (LDL-C) levels of >4.15 mmol/L, triglycerides (TG) levels of ≥2.3 mmol/L, and high-density lipoprotein cholesterol (HDL-C) levels of <1.2 mmol/L.

Samples were stored at 4˚C for further molecular analysis. All samples were stored after proper labeling with identification numbers given to the subjects.

DNA extraction of these samples was done via the FavorPrep™ Blood Genomic DNA Extraction Kit marketed by Favorgen R (Cat No: Kit# FABGK001).

In this study, this stored DNA was taken for analysis of a novel SNP.

The threshold probability for rejecting the null hypothesis type I error rate was 0.05.

Study subjects

A total of 150 diabetic dyslipidemia patients and 150 healthy controls were included in this study.

Inclusion criteria

Newly diagnosed type 2 diabetic patients with dyslipidemia, aged 25–70 years, and both genders.

Exclusion criteria

Type 1 diabetes mellitus patients, gestational diabetes patients, type 2 diabetes mellitus patients with other complications, patients on lipid-lowering therapy, and patients suffering from dyslipidemia-associated diseases, i.e., hypothyroidism, Cushing syndrome, polycystic ovarian syndrome, chronic liver disease, and cardiovascular diseases.

Molecular analysis

Gel electrophoresis

DNA was analyzed both qualitatively and quantitatively using agarose gel electrophoresis. To prepare 1% agarose gel, 0.8 g of powdered agarose was dissolved in 40 mL of 1x TBE (0.89 M Tris-Borate and 0.032 M EDTA). Gel casting was preceded by the addition of 2–4 µL of ethidium bromide (EtBr) dye. The extracted DNA samples were visualized on a gel documentation system (BioRad).

Primer designing

The sequence of the selected SNP was retrieved from the National Centre for Biotechnology Information (NCBI). The sequence was downloaded from the biological database "Ensembl". The SNP primers were generated using the bioinformatics web application tool "Primer 1". Primers specific to Tetra-ARMS PCR were developed. The sequence of the forward outer primer was 5´ACAGGGCCGAGCTGTTCGCACA3´, the reverse outer primer was 5´GCGTGACCTCCACCTTCAGCAAACG3´, the forward inner primer was 5´CCTTGCTCATCTCCTGCCTCAGGCC3´, and the reverse inner primer was 5´TGTCACCCAGGGCTCACCCCTGATA3´.

Data analysis

Demographic and biochemical data were analyzed using SPSS version 26. The Student independent t-test was used to calculate the mean±standard deviation of continuous variables between two groups. DNA samples were analyzed on 1% agarose gel, and TETRA-ARMS polymerase chain reaction was performed to amplify the *rs121912724* A>C polymorphism in the *APOA1* gene. Fisher's exact test and Cochran-Armitage tests were applied to find the association of the polymorphism with the disease. The correlation between polymorphism and HDL, TG, and LDL levels was determined using the Pearson correlation test.

Table 1. Comparison of Mean±SD of lipid profile and age between two groups

Variables	Diabetic dyslipidemia patients	Healthy controls	p
Age	52.0±10.80	44.14±15.58	0.000**
Serum HDL	0.79±0.25 mmol/L	1.30±0.37 mmol/L	
Serum LDL	2.9847±1.08 mmol/L	2.31±0.70 mmol/L	
Serum TG	3.22±1.75 mmol/L	1.43±0.60 mmol/L	

**: Highly significant. SD: Standard deviation; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglycerides.

Results

The mean age of diabetic dyslipidemia cases was 52±10.80 years, and that of healthy controls was 44.14±15.59 years. Out of 300 subjects, 37% were females, and 63% were males. Among 150 cases, 35% were females, and 65% were males, while in 150 healthy controls, 39% were females, and 61% were males. A significant difference was observed between levels of serum HDL, serum LDL, and serum TG in cases and controls, where the p-value was 0.000 (p≤0.05), as shown in Table 1.

The *rs121912724* polymorphism in the *APOA1* gene involves an A>C transition according to the SNPdb database, with allele C considered less common and the risk allele in this study. The genotypic distribution for homozygous AA was 275, heterozygous AC was 24, and homozygous CC was 1 (n=300). There was an insignificant difference observed in genotypic distribution between group I and group II for AA, AC, and CC. The AA genotype was the most common in both group I and group II compared to the AC and CC genotypes. There was also no association of the CC genotype of *rs121912724* with the development of diabetic dyslipidemia, and the AA homozygous genotype was displayed as protective (Table 2).

An insignificant correlation coefficient (r) value was observed between the *rs121912724* polymorphism and levels of HDL-C, LDL-C, and TG in the diabetic dyslipidemia group I (Table 3). The p-value was greater than 0.05 and was observed as insignificant.

Table 2. Association of *rs121912724* with diabetic dyslipidemia

Groups	Distribution of *rs121912724*			Cochran Armitage trend test	Fisher Exact value
	AA	AC	CC		
Diabetic dyslipidemia patients (n=150)	137	12	1	X^2=0.1554 0.6935	F=257 0.586
Healthy controls (n=150)	138	2	0		

Table 3. Correlation between *rs121912724* polymorphism and levels of HDL-C, LDL-C and TG

	HDL-C	LDL-C	TG
Diabetic dyslipidemia patients			
rs121912724			
Pearson correlation coefficient	-0.152	0.052	-0.057
Sig. (2-tailed)	0.063	0.530	0.486
n	150	150	150
Healthy controls			
rs121912724			
Pearson correlation coefficient	0.150	0.112	0.002
Sig. (2-tailed)	0.067	0.171	0.076
n	150	150	150

**: Correlation is significant at the 0.01 level (2-tailed). HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglycerides; Sig.: Significant.

Discussion

The International Diabetes Federation stated a 26.7% prevalence of diabetes in Pakistan in 2022 [14]. The genetic predisposition associated with dyslipidemia in type 2 diabetes is not well documented in the Pakistani population. This study found a novel single nucleotide polymorphism (SNP), not previously documented in diabetic dyslipidemia patients in Pakistan. This study showed that *rs121912724* with genotype AA was not associated with diabetic dyslipidemia, as the p-value was less than 0.005. Moreover, an insignificant correlation was observed between deranged levels of HDL-C, LDL-C, TG, and AA+AC genotypes. A study reported an association of rs5072 in the *APOA1* gene with dysregulated TG metabolism and increased susceptibility to dyslipidemia in schizophrenia patients [15]. The *APOA1/C3/A4/A5-ZPR1-BUD13* gene cluster was studied in dyslipidemia patients and found an association between polymorphism of the gene cluster and dyslipidemia [16]. Hypertriglyceridemia was observed as the most common abnormality in diabetic dyslipidemia, either due to poor clearance or increased production [17]. These studies augment the generation of a pool of genetic data in our population to understand the molecular etiology of dyslipidemia. In the current study, an equal frequency of the AA genotype in cases and controls has proposed a protective role of *rs121912724* against diabetic dyslipidemia. A previous study on apolipoprotein AI transgenic diabetic mice suggested the beneficial impact of an increased ratio of *APOA1/HDL* in diabetic people having reduced cardiovascular disease [18]. A study conducted on 135 type 2 diabetic patients reported an association of 83 C>T polymorphism in the *APOA1* gene with deranged levels of TG, HDL-C, and LDL-C, and predicted susceptibility to myocardial infarction [19].

The genetic data obtained from this research offered significant insights and expanded our comprehension of the molecular mechanisms and genetic predisposition to diabetic dyslipidemia in our population. This information enriched our knowledge base, enabling more thorough and detailed assessments of individual susceptibility to this condition. The insignificant association observed in this study may be due to the small sample size and needs to be studied in a larger population to gain better insight. Moreover, ethnicity is an important factor to be taken into account for polymorphism studies, as it contributes to the inconclusive effect of genotype.

Conclusion

The study concludes an insignificant association of *rs121912724* in the *APOA1* gene with diabetic dyslipidemia and a negative correlation between the SNP and levels of HDL-C, TG, and LDL-C.

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Ethics Committee Approval: The study was approved by The Army Medical College Ethics Committee (No: ERC/ID/271, Date: 21/06/2023).

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