Assessment of Apoe Gene Variants and Apob-100 R3500q Mutation as Genetic Risks for Dyslipidemia: A Case-Control Study

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Abstract
Background: Dyslipidemia is defined as an abnormally high level of various lipids in the blood. It is considered a major risk for atherosclerosis and coronary artery disease. Genetic susceptibility can have a significant influence on the development and progression of dyslipidemia. ApoB-100 R3500Q mutation and ApoE variants are among those genetic risks for dyslipidemia. This study aims to assess the possible contribution of ApoB and ApoE variants on lipid profile among a group of early-onset ischemic heart disease (IHD) patients in comparison to a group of controls. Methods: Forty patients with dyslipidemia and early-onset IHD without chronic conditions likely to cause derangement of lipid levels were recruited to this case-control study along with 20 disease-free controls. Basic demographic and clinical features along with lipid profile changes of the recruited individuals were analyzed and correlated with ApoB R3500Q mutation and ApoE variants in both groups. Results: The majority (80%) of participants were males. Hypertension showed a significant association with abnormal lipid profile among the patients, unlike family history of dyslipidemia, IHD or stroke, smoking status, and parental consanguinity, where no significant association was observed. ApoB R3500Q mutation was detected in a heterozygous state in one IHD patient only. ApoE variants were reported as follows: e3/e3 in 81.7% of recruited individuals while 10% have e3/e4 variant and 8.3% have e2/e3 variant. None of these variants showed a significant correlation with most clinical and lipid profile abnormalities. A noticeable proportion (25-30%) of the controls had marginally increased TC and TG levels respectively, while 60% of the controls had borderline high VLDL levels, which warrants further evaluation. Conclusions: The studied ApoB and ApoE variations do not seem to be the major contributing factors for dyslipidemia and IHD among the recruited individuals. The unhealthy lifestyle or other genetic causes are possible culprits in the absence of chronic medical conditions, which requires the application of certain preventive/therapeutic measures for the community.

Keywords: ApoB-100 R3500Q, ApoE variants, dyslipidemia, HDL, hypercholesterolemia, ischemic heart disease, LDL, triglycerides

1. Introduction

Dyslipidemia, defined as an abnormally high levels of lipids, which typically includes total cholesterol (TC), lipoproteins, chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and apolipoproteins, and low level of high-density lipoprotein (HDL), [1] is one of the main risk factors for the development and progression of cardiovascular diseases (CVDs). [2]

It is a very common condition with an increasing frequency throughout the world. There are over three million adults throughout the United States and Europe that currently have a diagnosis of dyslipidemia, and that number continues to rise at a drastic pace. Although it does not typically lead to critical symptoms by itself; however, having the underlying pathology will often lead to serious illnesses that may ultimately lead to death. [3]

Dyslipidemia is a complex disease and is a major risk factor for adverse cardiovascular events as it is known to promote atherosclerosis. [4]

CVDs remain the leading cause of disease burden in the world. There has been a noticeable steady increase in CVDs prevalence and deaths in the last few decades. Prevalent cases of total CVD nearly doubled from 271 million in 1990 to 523 million in 2019, and the number of CVD deaths steadily increased from 12.1 million in 1990, reaching 18.6 million in 2019. CVD burden continues its decades-long rise for almost all countries outside high-income countries, and alarmingly, the age-standardized rate of CVD has begun to rise in some locations where it was previously declining. [5]

Almost one-fifth (18%) of global mostly non-fatal stroke events, and about 56% of global heart disease are attributable to high cholesterol levels. This amounts to about 4.4 million deaths (7.9% of the total) and 2.8% of the global disease burden. People with high total cholesterol have approximately twice the risk of heart disease as people with optimal levels. [6]

Dyslipidemia is classified into primary and secondary types. Primary dyslipidemia is basically inherited or familial and caused by single or multiple gene

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mutations that result in either overproduction or defective clearance of triglycerides and cholesterol; Table (1). Secondary dyslipidemia is caused by unhealthy lifestyle factors and acquired medical conditions, including some endocrine, renal, liver, and gall bladder problems as well as social habits (smoking, alcohol consumption) and drugs. It accounts for approximately 30-40% of all dyslipidemias. [7]

Table 1. Frederickson's classification for primary dyslipidemia based on the pattern of lipoproteins on electrophoresis or ultracentrifugation [5]

Phenotype	Increased lipoprotein	Plasma TC level	Plasma TG level	Atherogenesis	Relative frequency (%)
I	Chylomicrons	Normal or ↑	↑↑↑↑	+	<1
IIa	LDL	↑↑	Normal	+++	10
IIb	LDL and VLDL	↑↑	↑↑	+++	40-45
III	IDL and remnants	↑	↑↑↑	+++	<1
IV	VLDL	Normal or ↑	↑↑	+	45
V	Chylomicrons and VLDL	↑ or ↑↑	↑↑↑↑	+	6

IDL: intermediate-density lipoprotein; LDL: low-density lipoprotein; TC: total cholesterol; TG: triglycerides; VLDL: very low-density lipoprotein

The most common primary dyslipidemias are types IIA, IIB, and IV. Type I and type III hyperlipoproteinemia (HLP) are extremely rare in pediatric patients, and type V is uncommon. [8]

Genetic causes of dyslipidemia can be monogenic or polygenic. Monogenic dyslipidemias are caused by rare DNA variants that have a strong impact on phenotype. Polygenic dyslipidemias are due to multiple common genetic variants. Currently, 25 monogenic dyslipidemias have a well-established molecular genetic basis. These tend to be characterized by extreme levels of LDL, TG, HDL, and/or other lipids. [9]

The major portion of inter-individual variation in plasma lipids is polygenic, attributable to sequence variation in various loci. Both ApoB and ApoE are considered major genes affecting the circulating lipid levels. [10]

ApoB is encoded by a gene that has been localized to the short arm of chromosome 2 (2p24.1). Apolipoprotein B (ApoB) is a large, amphipathic glycoprotein that plays a central role in human lipoprotein metabolism. [11] ApoB-100 is produced in the liver. This protein is a building block of VLDLs, IDLs, and LDLs. These related molecules all transport fats and cholesterol in the bloodstream. ApoB-100 allows LDLs to attach to specific receptors on the surface of cells, predominantly in the liver. Once attached, the receptors transport LDLs into the cell, where they are broken down to release cholesterol. The cholesterol is then used by the cell, stored, or removed from the body. [12]

One of the most common single-site mutations in the human ApoB gene, namely the ApoB-100 R3500Q, leads to diminished affinity for its receptor and results in mild to severe hypercholesterolemia and an increased risk for early-onset atherosclerosis. [13]

It is widely accepted that the average white population frequency of the R3500Q mutation is about 1:500-1:700. [14]

The ApoE gene is located on chromosome 19. Apolipoprotein (Apo) E is a 299-residue protein which functions as a key regulator of plasma lipid levels. [15] ApoE glycoprotein plays an important role in metabolism, transport, and redistribution of lipoprotein molecules that carry cholesterol and other lipids and mediates the uptake of chylomicrons, VLDL and IDL. [16]

It is a polymorphic protein with different isoforms. The three most common ApoE variants are designated as the e2, e3, and e4 alleles. ApoE e3 (112 Cys, Arg 158) is the wild-type allele; ApoE e4 (rs429385) (Arg 112, Arg 158) is associated with an increase in LDL-cholesterol levels and thus a higher cardiovascular risk compared to ApoE3. ApoE4 is also associated with Alzheimer's disease. ApoE e2 (rs7412) (Cys 112, Cys 158) is linked to an increased risk for early CVD and hyperlipoproteinemia type III but is also associated with a mild decrease in LDL-cholesterol levels and thus has a mild protective effect. [17] Additionally, ApoE e2 alleles are also associated with longevity. [18] ApoE e4 allele is a chief reason to instigate coronary artery disease (CAD) while the ApoE e2 allele is beneficial to mitigating the CAD risk. [19] The distribution of these alleles is varied among different ethnic populations. At a global level, higher frequencies of the ApoE e2 allele were observed in Africa while Indian and Asian populations showed the highest frequencies of E3 allele while ApoE e4 showed a significant increasing cline in North European populations. [20]

Zygosity status of both ApoE and ApoB is also important, as heterozygous individuals may be less symptomatic than homozygotes and different combinations of ApoE alleles may be linked to various clinical outcomes. [21]

This study aims to assess the possible contribution of different ApoE alleles and ApoB-100 R3500Q mutation as genetic risk factors for dyslipidemia by determining their status among patients with dyslipidemia and early-onset CAD patients in comparison to disease- and symptom-free controls and to correlate these genetic risk factors with clinical and biochemical characteristics of the enrolled individuals.

2. Methods

This is a case-control study that recruited 40 supposedly genetically predisposed unrelated Iraqi

patients with dyslipidemia and early-onset CAD admitted to the Cardiac Care Unit (CCU) at Ibn Al-Nafees Teaching Hospital in Baghdad, Iraq.

Genetic predisposition for IHD is considered in younger and at higher risk CAD patients who do not have secondary causes of dyslipidemia. Therefore, male patients older than 50 years, female patients older than 55 years, those with known thyroid problems, diabetes mellitus, other endocrine disorders, chronic kidney diseases, or those on lipid-lowering drugs were excluded from the study.

In addition, 20 disease-free and symptom-free individuals from the general population who are age and sex-matched were recruited as a control group.

Findings of a thorough medical and family histories, physical examination, and results of investigations (as indicated) were recorded for each participant.

Lipid profile and molecular genetic testing for ApoE gene variants and ApoB R3500Q mutation status were performed for all patients and controls.

Lipid profiles (total cholesterol, triglycerides, and HDL) were measured from a peripheral blood sample using the automated biochemistry analyzer Spin200E® while LDL and VLDL were calculated using the Friedewald formula. Different lipid ratios (TG/HDL, TC/HDL, and LDL/HDL ratios) were also calculated accordingly.

The molecular diagnosis included DNA isolation (using GENXTRACT Blood DNA Extraction System® (ViennaLabs Diagnostics), followed by PCR-amplification using biotinylated primers. Hybridization of amplification products to test-strips containing allele-specific oligonucleotide probes that are immobilized as an array of parallel lines was performed. The bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and color substrates. The working protocol followed the manufacturer's instructions. [22] The materials were supplied from one ready-to-use kit (CVD-StripAssay, Vienna Labs Diagnostics GmbH – Austria).

Statistical analysis of the obtained results was performed using Microsoft Excel ver. 2016 and IBM-SPSS version 28. Chi-square, Fisher's exact t-tests and multivariate analysis were used. A p-value of <0.05 was considered statistically significant.

This study was approved by the ethical committee of the Dept. of Pathology and Forensic Medicine, College of Medicine, University of Baghdad (Issue No. 168 at 28.12.2020). All patients and controls have given their verbal consent to participate in this study and agreed to publish their data provided that their identities remain anonymous.

3. Results

Age and sex distributions

The age of patients ranged between 20-55 years, with a mean ± SD of 45.8 ± 8.1 years, while that of the controls ranged between 37-55 years with a mean ± SD of 46.6 ± 5.8 years. There was no significant difference in age distribution between both groups (p=0.72).

The majority of recruited individuals among both groups were males [31/40 (77.5%) for patients and 17/20 (85%) for controls]. There was no significant difference in sex distribution between both groups (p=0.49).

Clinical data

Family history of dyslipidemia, family history of MI or stroke, smoking status, and parental consanguinity did not show a significant difference between patients and controls (p>0.05); only hypertension showed a statistically significant difference between the two groups (p<0.001); Table (2). It is worth mentioning that all females in both groups (9 patients and 3 controls) were non-smokers.

Table 2: Comparison of some clinical data between patients and controls

Variables		Patients		Controls		
		No.	%	No.	%	P value*
Family history of dyslipidemia	**Negative**	**23**	**57.5%**	**9**	**45%**	**0.36**
	Positive	17	42.5%	11	55%	
Family history of MI or stroke	Negative	9	22.5%	4	20%	0.55**
	Positive	31	77.5%	16	80%	
Smoking status	No	21	52.5%	15	75%	0.09
	Yes	19	47.5%	5	25%	
Hypertension	No	17	42.5%	20	100%	0.0001**
	Yes	23	57.5%	0	0.0%	
Parental Consanguinity	Negative	23	57.5%	11	55%	0.85
	Positive	17	42.5%	9	45%	

*Chi-Square test; **Fisher's exact test

Regarding lipid profile among participants, the means of serum TC, TG, LDL, and VLDL were significantly higher among patients in comparison to controls (p<0.001), while HDL was significantly lower among patients in comparison to controls (p=0.0001); Table (3). High-risk levels of some lipid profile were found in the patients' group as follows: 28(70%) for TC and TG, 1(2.5%) for HDL, 22(55%) for LDL, 27(67.5%) for VLDL, 34(85%) for TG/HDL, 22(55%) for LDL/HDL and 14(35%) for TC/HDL ratios. A desirable level in the patient's group was only limited to a few of them: 2(5%) for TC, 20(50%) for HDL, 8(20%) for LDL, 6(15%) for LDL/HDL, and 4(10%) for TC/HDL ratios while it was 0(0%) for TG

and VLDL, and TG/HDL; Table (3).

Table 3. Lipid profile results of all participants (patients and controls)

Serum level (mg/dL)		Patients			Controls			P value
		No.	%	Mean ±SD (mg/dL)	No.	%	Mean ±SD (mg/dL)	
Serum TC	Desirable (<200)	2	5%	269.38 (45.34)	15	75%	191.70 (14.96)	0.0001
	Borderline high (200-239)	10	25%		5	25%		
	High risk (>240)	28	70%		0	0.0%		
Serum TG	Desirable (<150)	0	0.0%	224.15 (52.24)	14	70%	134.85 (23.715)	0.0001
	Borderline high (150-199)	12	30%		6	30%		
	High risk (200-499)	28	70%		0	0.0%		
Serum HDL	Desirable (>45)	20	50%	48.55 (7.01)	19	95%	58.00 (8.253)	0.0001
	Borderline (35-45)	19	47.5%		1	5%		
	High risk (<35)	1	2.5%		0	0.0%		
Serum LDL	Desirable (60-130)	8	20%	171.0 (41.90)	20	100%	106.65 (18.242)	0.0001
	Borderline (130-159)	10	25%		0	0.0%		
	High risk (160-189)	22	55%		0	0.0%		
Serum VLDL	Desirable (<30)	0	0	44.63 (10.52)	8	40%	31.80 (5.521)	0.0001
	Borderline (30-40)	13	32.5%		12	60%		
	High risk (>40)	27	67.5%		0	0.0%		
TG/HDL ratio	Desirable (<3)	0	0	4.69 (1.13)	19	95%	2.81 (0.66)	0.0001
	Borderline (3.1-3.8)	6	15%		1	5%		
	High risk (>3.8)	34	85%		0	0		
LDL/HDL ratio	Desirable (<2.5)	6	15%	3.64 (1.18)	19	95%	1.89 (0.5)	0.0001
	Borderline (2.5-3.3)	12	30%		1	5%		
	High risk (>3.3)	22	55%		0	0		
TC/HDL ratio	Desirable (<4)	4	10%	5.67 (1.33)	18	90%	3.37 (0.57)	0.0001
	Borderline (4-5)	22	55%		2	10%		
	High risk (>5)	14	35%		0	0.0%		

Molecular analysis of ApoB and ApoE genes

Only one (2.5%) patient was identified to have a heterozygous ApoB-100 R3500Q mutation among the patient's group, while none (0%) have this mutation among the controls. This patient is a 50-year-old male with a positive family history of dyslipidemia and ischemic heart disease. His serum TC, TG, LDL, HDL, and VLDL were 290, 235, 198, 45 and 47 mg/dL respectively. His lipid ratios were 5.22, 6.44, and 4.40 for TG/HDL, TC/HDL, and LDL/HDL respectively. All were in high-risk or borderline high range. He was admitted to the CCU with acute MI.

Among the 60 participants and in regard to ApoE status, 49 (81.7%) were homozygous for E3/E3 variant [35/40 (87.5%) patients and 14/20 (70%) controls], 5 (8.3%) participants [3/40 (7.5%) patients and 2/20 (10%) controls] had heterozygous E2/E3 variant, while heterozygous E3/E4 ApoE variants were identified among 6 (10%) participants [2/40 (5%) patients and 4/20 (20%) controls]. None (0%) of the studied individuals showed homozygous E2/E2, E4/E4, or heterozygous E2/E4 alleles. There was no significant difference in distribution of ApoE variants between patients and controls (p=0.165); Table (4).

Table 4. Distribution of different ApoE variants between the studied groups

ApoE Status	Patients No. (%)	Controls No. (%)	Total No. (%) of each ApoE variant
E2/E3	3 (7.5%)	2 (10.0%)	5 (8.3%)
E3/E3	35 (87.5%)	14 (70.0%)	49 (81.7%)
E3/E4	2 (5%)	4 (20%)	6 (10%)
Chi-Square=3.6, p-value = 0.165			

By running multinomial logistic regression analysis for ApoE based on a cases category with E3/E3 used as a reference, none of the given variables showed significant association with heterozygous ApoE gene polymorphism [e2/e3 and e3/e4]. (p>0.05); Table (5).

Table 5: Logistic regression analysis for ApoE based on cases category

Variables	Likelihood Ratio Tests		
	Chi-Square (X^2) value	Degree of freedom (Df)	P value
Sex	3.616	2	0.164
Family history of dyslipidemia	1.053	2	0.591
Family history of MI	1.548	2	0.461
Smoking status	4.170	2	0.17
Hypertension	1.826	2	0.401
Ischemic heart disease	5.405	2	0.067
Parental consanguinity	5.888	2	0.053

To assess the probable contribution of ApoE variants on abnormal lipid profiles, a multivariate analysis was performed for both patients and controls. It showed no statistically significant association between ApoE variants with most types of lipid profile parameters. HDL as well as TG/HDL and LDL/HDL ratios showed a statistically significant difference between e2/e3 and e3/e4 among the controls; Table (6).

Table 6. Correlation between *ApoE* variants and various lipid levels between patients and control groups

Lipid profile		Patients' *ApoE*			P value	Controls' *ApoE*			P value
		E2/E3	E3/E3	E3/E4		E2/E3	E3/E3	E3/E4	
		Count	Count	Count		Count	Count	Count	
TC	Desirable	0	2	0	0.9	1	12	2	0.2
	Borderline high	1	8	1		1	2	2	
	High risk	2	25	1		0	0	0	
TG	Desirable	0	0	0	0.24	1	10	3	0.8
	Borderline high	2	10	0		1	4	1	
	High risk	1	25	2		0	0	0	
HDL	Desirable	2	18	0	0.62	1	14	4	0.009*
	Borderline	1	16	2		1	0	0	
	High risk	0	1	0		0	0	0	
LDL	Desirable	0	7	1	0.34	2	14	4	--
	Borderline high	2	8	0		0	0	0	
	High risk	1	20	1		0	0	0	
VLDL	Desirable	0	0	0	0.64	1	5	2	0.83
	Borderline	2	11	0		1	9	2	
	High risk	1	24	2		0	0	0	
TC/HDL	Desirable	0	4	0	0.93	1	13	4	0.12
	Borderline high	2	19	1		1	1	0	
	High risk	1	12	1		0	0	0	
TG/HDL	Desirable	0	0	0	0.57	1	14	4	0.009*
	Borderline high	1	5	0		1	0	0	
	High risk	2	30	2		0	0	0	
LDL/HDL	Desirable	0	6	0	0.87	1	14	4	0.009*
	Borderline high	1	10	1		1	0	0	
	High risk	2	19	1		0	0	0	

TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein.
* = A statistically significant association

4. Discussion

Dyslipidemia is a term that encompasses various genetic and acquired disorders that describe elevated lipid levels within the body. It is very common with an increasing frequency throughout the world. To lower morbidity and mortality rates associated with it, it is critical to establish an early diagnosis and prevent the progression and complications of this condition. [3]

The prevalence of dyslipidemia and therefore CVDs varies from one region of the world to another. It is common among the Iraqi population that is evident from various official reports and studies from different cities. CVDs represent the main causes of hospital admission and account for around 40% of all causes of death in the country. [23]

One study showed that the prevalence of any type of dyslipidemia among young adults (20-40 years) was 75% and that of elevated TC/HDL ratio was 61%, [23] while triglyceridemia was reported in another Iraqi study to be above 41% based on American NCEP III criteria; [24] Similar findings were reported from another Iraqi study. [25]

However, these studies are cross-sectional local studies limited to one city or one hospital and have correlated dyslipidemia to non-genetic risk factors (e.g., male sex, hypertension, diabetes) or disease outcomes e.g., ischemic heart disease.

The study of genetic risk factors is an attractive field as it enables therapeutic as well as preventive programs to be applied more effectively. Yet, determination of these risky genes for dyslipidemia and CAD is laborious, costly, and cannot be performed on a routine basis, especially in resource-limited countries.

Only a few studies tried to assess the role of some genetic risk factors in Iraq on atherosclerosis and CAD patients, [26, 27] but none have studied the risky genes on lipid profile disturbances.

Following the reported association between ApoB R3500Q and ApoE polymorphism with CVDs and CVD risk factors like dyslipidemia, several studies worldwide have been carried out to study their role, geographical distributions and the prevalence of those genes in different populations. [14]

For this reason, this study was performed to assess the possible contribution of different ApoE alleles and ApoB R3500Q mutation as genetic risk factors on dyslipidemia by determining their status among a group of patients with dyslipidemia and premature CAD patients in comparison to disease- and symptom-free controls from the general population and to correlate these genetic risks with clinical and biochemical characteristics of the enrolled individuals.

The premature onset of dyslipidemia complications, namely hypertension and CAD as well as positive

family history in some patients when secondary causes of dyslipidemia have been excluded may imply a genetic predisposition in those individuals. Such cases were selected for this study.

The premature (early-onset) CAD is defined as the first manifestation of CAD in male patients less than 55 years old and in female patients less than 65 years old. [28]

In this study, the mean age of the patients was 45.8 ± 8.1 years, and the mean age of controls was 46.6 ± 5.8 years.

Nowadays, all age groups, even adolescents, are more likely to develop CVD risk factors at an early age as due to modernization and changes in the family structure. [29]

In this study, males represented the majority of cases among dyslipidemia patients, and this was comparable to other studies that found males have higher rates of dyslipidemia in comparison to females. [30] Moreover, it was suggested that, when untreated, dyslipidemia is a higher risk factor for stroke and stroke-related mortality in men than in women. [31]

Family history of dyslipidemia, IHD or stroke, smoking status, and parental consanguinity all showed a non-significant difference between patients and controls. However, hypertension showed a significant association with the patients' group in comparison to the controls. The latter result supports other reports from Iraq and various regional studies. [32, 33] Dyslipidemia and lipid peroxidation are thought to be important determinants of atherosclerosis that leads to hypertension and ultimately to CAD. [34]

Tobacco has been recognized as a major risk factor for the development of IHDs and may lead to alteration of the normal plasma lipoprotein pattern. It has long been established that tobacco contains nicotine and that it has a considerable influence on the increasing levels of lipids in the blood. [35] A decreased level of HDL and increased levels of TC, LDL, and TGs has been observed in smokers as compared to non-smokers. [36] Another study concluded that LDL levels increased in smokers relative to nonsmokers, consistent with findings in other populations. [37]

In this study, there were 24 smokers; all were males (19 patients and 5 controls). Smoking significantly affects TG/HDL, LDL/HDL and TC/HDL ratios between patients and controls; p-value <0.05.

The TG/HDL ratio may be a more favorable predictor of CVDs than lipid profile serum levels alone. [38] Evidence showed that an elevated serum TG/HDL ratio independently predicted a decrease in LDL particle size. [39]

Many studies suggested that TC/HDL ratio is also closely related to CVD. [40] Moreover, TC/HDL and TG/ HDL ratios may reflect a decrease in mature large-size HDL particles, which is related to anti-atherosclerosis. [41] LDL/HDL ratio is considered a risk indicator with greater predictive value than isolated parameters when used independently, particularly LDL. [42]

In the current study, TC/HDL ratios showed no significant association neither with the clinical parameters nor with various ApoE variants or ApoB mutation, which may point to a non-genetic cause of hyperlipidemia in the studied patients. However, TG/HDL and LDL/HDL ratio were significantly associated with various ApoE variants. This finding can only be interpreted with caution as the number of patients is limited.

The observation that 25-30% of the control group in this study have marginally increased levels of TC and TG and 60% of the controls have borderline high VLDL levels is not to be overlooked. Despite the small number of tested controls (n=20), those apparently healthy people had subclinical lipid disturbance. This is neither strange nor unique but clinically important and worth further investigations. In a recent Iraqi study, dyslipidemia was reported to be highly prevalent in apparently healthy Iraqi peoples with subnormal levels of HDL as found in 64.5% of participants [43]. Similar findings (30-40%) were reported to have some sort of lipid disturbances from different populations all over the world: 38% of American adults have high cholesterol; [44] the overall dyslipidemia prevalence in China is 31.2% [45]; hypercholesterolemia, hypertriglyceridemia, high LDL, and low HDL were 44.3%, 41.9% 75.9%, and 59.5%, respectively among Jordanian people; 33.7% of men and 30.6% of women from Kuwait and 33.5% from Oman were reported to have hypercholesterolemia; Saudi Arabia (54%) and in Iran (41.6%). [46]

Autosomal dominant familial defective apolipoprotein B-100 (FDB) is caused by mutations in and around the codon 3500 of the apolipoprotein B (Apo B) gene. ApoB-100 R3500Q mutation is the first ApoB mutation known to be associated with FDB and it is the most frequently reported Apo B mutation in several different populations. ApoB-100 R3500Q mutation is known to have wide geographic and population distribution changes; its average population prevalence is about 1:500–1:700 in North America and Central Europe. [47]

ApoB-100 R3500Q mutation accounts for 0.99–8.17% of the cases with hypercholesterolemia, and therefore, represents the most common single gene defect resulting in familial hypercholesterolemia (FH) identified in Bulgaria but the mutation was not detected in any of the patients diagnosed with FH in Russia. [48] In Lebanon, the R3500Q mutation was not observed in the general Lebanese population. [49]

In the current study, only one patient was heterozygote for this mutation, with gross lipid abnormalities and premature CAD. This mutation is likely be an important risk of his condition. In a previous Iraqi study, ApoB-100 R3500Q polymorphism was not detected among 102 MI Kurdish patients. [27]

Apolipoprotein E (Apo E), a major component of blood chylomicrons, VLDL, and HDL particles, has several alleles that differ by point mutations that are associated with a range of clinical phenotypes. [50] ApoE2, E3, and E4, represent >95% of ApoE alleles worldwide. In most populations, ApoE3 is the most common, followed by E4 and then E2. [51, 52] In this study regarding the frequency of apoE alleles, E3 dominated, with E4 being slightly more common than E2 among the controls. This was similar to what has been reported earlier among a group of Iraqi CAD patients in Baghdad [78.6% for E3/3, 14.3% for E2/3, 5.3% for E3/4, and 1.8% for E2/4 variants] [26] and in Kurdistan, where ApoE3/3 genotype frequency was (80.7%) followed by ApoE 2/3 (12.3%), and ApoE 3/4 (7%). [53]

These findings were nearly similar to the current study regarding the frequency of ApoE gene polymorphism. No significant difference in the distribution of ApoE variants was found between patients and controls (p=0.165).

This pattern of distribution and frequency is similar to what was reported by regional countries e.g. Qatar, which showed a prevalence of E3/E3 to be 74% and E2/E3 to be 5%, but they reported a higher prevalence for E3/E4 with 16%. [54]

ApoE e2-containing lipoproteins have delayed clearance, leading to up-regulation of LDLR that would result in lower levels of LDL, higher HDL and TGs levels. On the other hand, ApoE e4-containing lipoproteins are catabolized rapidly leading to down-regulation of LDLR that would result in increased hepatic cholesterol synthesis, higher LDL and TGs and lower HDL levels [10]. Although LDL does not contain ApoE, the isoforms influence its concentration and size along with many other factors including sex, age, and TG concentration. Smaller LDL is associated with higher cardiovascular risk and lower HDL concentration. ApoE e2 carriers had smaller LDL with normal HDL size, unlike apoE4 carriers who had a preponderance of smaller HDL. [55] Middle-aged healthy Arabian carriers of apoE2 had no small LDL compared with controls without apoE2; however, older subjects with apoE2 and CAD had smaller LDL [56]

In a recent meta-analysis studying the association between apolipoprotein gene polymorphisms and dyslipidemia, the correlations in the ApoE genotype (E2/E3, E3/E4) and dyslipidemia using the wild-type E3/E3 genotype as a reference, it demonstrated a significant difference in risk of dyslipidemia in carriers of all genotypes (E2/E3, E3/E4) compared with carriers of the E3/E3. [57] Therefore, it was concluded that the ApoE gene variants, other than E3/E3, can be considered to be closely associated with dyslipidemia.

The current study did not show such a significant association between ApoE variants with clinical or biochemical findings of lipid profile, except for HDL, TG/HDL, and LDL/HDL ratio with e2 and e4 alleles among the controls. This finding further suggests a probable overall non-genetic influence on lipid profile abnormalities among the studied individuals, which was also been reported by a previous Iraqi study that detected a non-significant difference in the allele frequencies of ApoE e4 between patients and controls, which is consistent with a large meta-analysis including more than 22,000 British patients, which also failed to document such a link. [27]

5. Conclusion

Despite the early onset and genetic susceptibility of the recruited IHD patients, it seems that ApoE variants and ApoB-100 R3500Q mutation have a non-significant effect on dyslipidemia in the studied sample. Most patients have either other genetic risks or non-genetic influences, mainly unhealthy lifestyle and unhealthy diet, which have led to dyslipidemia and premature IHD. The is supported by the observation of borderline high levels of certain lipids in about one-third of the control group, which warrants further investigation.

Limitations of the study

The small number along with a non-random selection of participants (patients and controls) makes generalization of the obtained results and conclusions on a population level in question. Thus, it is recommended to validate these observations on large-scale studies. In addition, testing only two genetic risks from a number of risky genes and genetic variants that have a cumulative effect on one condition can be tricky in interpretation.

Abbreviations

APO	Apolipoprotein
CAD	Coronary artery disease
CCU	Cardiac care unit
CVD	Cardiovascular disease
HDL	High-density lipoprotein
HLP	Hyperlipoproteinemia
IHD	Ischemic heart disease
LDL	Low-density lipoprotein
PCR	Polymerase chain reaction
TC	Total cholesterol
TG	Triglyceride
VLDL	Very low-density lipoprotein

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Declarations

Ethics approval and consent to participate

All recruited individuals (patients and controls) have given their verbal consent to participate in this study. This study was approved by the ethical committee of the Dept. of Pathology and Forensic Medicine, College of Medicine, University of Baghdad (Issue No. 168 at 28.12.2020).

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

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Authors' contributions

RKOA: collected the data, performed molecular analysis, and drafted the manuscript.
BMSA: conceptualized and designed the study, contributed to data analysis, drafting and finalization of the manuscript.

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