ORIGINAL ARTICLE

CONTENTS

The impact of estrogen receptor β gene polymorphisms on the atherogenic index and coronary artery disease in Iraqi population

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ABSTRACT

Aim: To study the impact of the estrogen receptor beta polymorphisms rs4986938 (AluI) and rs1256049 (RsaI) on ischemic heart disease as well as the atherogenic index in Iraqi population.

Materials and Methods: A case-control research study was conducted on a sample of Coronary artery disease patients in addition to a group of normal individuals. Serum lipid concentrations were measured in the participants, and the ER gene (rs1256049, rs4986938) was genotyped by PCR- RFLP.

Results: Atherogenic serum lipid concentrations increased significantly in Coronary artery disease patients relative to the control group. Genotyping of the estrogen receptor β gene forrs4986938 SNP revealed a significant (OR=2.79, P=0.000) elevation of the GA genotype carriers in Coronary artery disease versus the control groups. The genotype analysis of thers1256049 SNP failed to exhibit a significant variation. Atherogenic index and serum lipid concentration markedly elevated with these polymorphisms.

Conclusions: The polymorphisms of ESRβ rs4986938, (not for rs1256049) was associated with risk of Coronary artery disease and implicated in changes of serum lipid concentrations and atherogenic index in Iraqi population. Additional studies of ESRβ genetic variation and risk of CVD are warranted.

KEY WORDS: estrogen receptor beta, coronary artery disease, atherogenic index, ischemic heart disease, Polymorphisms

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INTRODUCTION

Coronary artery disease (CAD) is the main cause of morbidity and mortality in the developed world and developing cities [1-2]. It is responsible for about 1 in every 3 deaths in people over the age of 35. Iraq ranks 20 in the world at age-adjusted death rates [3]. Cardiovascular disease is the first cause of disease-related death in Iraq [4]. CHD rate in our country is alarming and is mostly linked to the grouping of cardiovascular risk factors [5]. The primary cause of CAD is atherosclerosis that starts with coronary arterial streaks of fatty tissue lesions that restrict the coronary arteries or its branches impeding myocardial perfusion [6-7]. Coronary artery disease is a disease that has multi-factorial features, exaggerated by both environmental and genetic factors. It corresponds with a polygenic; many genes with different alleles that may have mild to moderate effects [8]. Studies highlighted the association of numerous gene polymorphisms with CHD [9-10]. The ER βgene, which is present in the layers of the vessel wall in the vasculature, is one of the genes suspected of being implicated in the cause of CHD [11]. ERβ is a protein of 530 amino acids [12]. ERβ is known to be expressed in both endothelial and VSMC of arteries [13]. The gene of ERβ is located in the 14q23.2-q23.3. It comprises 17 exons and 16 introns [14]. There are two polymorphisms in ERβ the geners1256049, rs4986938; they are located in exon 5, 8 respectively [15]. The first, rs125649G>A, is a silent polymorphism that replaces guanine with adenine [16], the second, rs4986938 G>A (replaces guanine with adenine) in non-coding area of 3 untranslated region of exon8 in the ERβ gene. Little attention was focused on estrogen receptor β polymorphism in CAD. Little emphasis was paid to estrogen receptorβ polymorphism. Few studies have found that these SNPs are linked to CAD in particular populations [17-18]; however, the association of ERβ gene polymorphisms with CAD has not been examined in Iraq. The current investigation was carried out to confirm the effect of estrogen receptor polymorphisms in the gene on the atherogenic indexes and coronary arteries in the Iraqi population.

MATERIALS AND METHODS

STUDY INDIVIDUALS

A case-control research study was conducted on a group of CVD patients as well as a group of individuals who were healthy. Serum lipid concentrations were measured in the participants, and the ER gene (rs1256049, rs4986938) was genotyped. Participants completed an extensive questionnaire that included age, gender, past family medical conditions, and other pertinent information. BMI values were calculated using weight and height measurements. It is worth noting that the Al-Najaf Centre for vascular surgery and catheterization of the cardiovascular system is a national institution that receives patients from all across Iraq, including the northern region, middle, and south. As a result, the current demographic sample is representative of the Iraqi population. All subjects provided informed consent. From March to August 2022, the biochemical and genetic studies were carried out in the laboratory of the Department of Biochemistry, the College of Medicine in Kufa, Iraq. Ethical Committee from the Kufa faculty of medicine authorized the study.

PATIENTS GROUP

It included 150 CAD patients who had undergone coronary surgery and performed cardiac catheterization at the Al-Najaf center. The patient's age was 52.06 ± 8.88 (Mean ± SD) years. Specialist physicians diagnosed the patients. Patients with unstable angina or myocardial infarction (MI) were eligible if their angiography indicated 70% or more obstruction of the coronary arteries or its branches, and their ages ranged from 30 to 75 years. The criteria for being excluded were as follows: Use of contraceptive pills and hormone or replacement treatment in the month preceding the study, family history of hypercholesterolemia, cancer, connective tissue disease and Diabetes mellitus patient.

CONTROL GROUP

It was made up of 150 healthy people. They were chosen from the general community who came to the hospital for a routine check; the ages were 53.25±8.83 years. The following were the inclusion criteria:

- no past medical history of CHD,
- no history in the family of CHD,
- no past medical history of DM,
- matched to patients with regard to age, sex, and geographical distribution.

BIOCHEMICAL MEASUREMENTS

Fasting serum lipid (cholesterol, triglycerides, and HDL-C) concentrations were measured. They were measured in accordance with the manufacturer's specifications. VLDL-C and LDL-C were calculated in an indirect manner. The atherogenic index in plasma (AIP) and the triglyceride/HDL-C ratio were also determined.

GENOTYPIC MEASUREMENTS

For DNA analysis, blood samples from CAD patients and control groups were obtained in EDTA-anticoagulant tubes. A small kit (FAVORGENTM Total DNA Extraction) was used to extract the DNA. RFLP was used to genotype the ERβ gene for rs4986938, rs1256049 SNPs using particular restriction enzymes (AluI), (RsaI) respectively and then analyzed by gel electrophoresis.

The primers were chosen as previously reported [19], those in favor of SNP were:

For rs4986938 were:
Forward primer: 5-GTGTGTGGTGGGACACAGAG-3
Reverse primer: 5-AGGCCATTGAGTGTGGAAAC-3
Those for rs1256049 SNP were:
Forward primer: 5-TTCTGAGCCGAGGTCGTAGT-3
Reverse primer: 5-TGAATCCTTGGACCCAACTC-3

The amplification process included an overall volume of 25 µl and was made up of 12.50 µl GoTaq® G2 Green's Masters Mix [28], 0.8 µl each of the primer, enough nuclease-free water to fill the whole volume, and 5 µl of the genomic DNA template. For the rs4986938 G/A, the PCR reaction program was as follows: 5 minutes at 95°C, 35 cycles of 95°C for 50 s, 63°C for 50 s, 72°C for 50 s, and final elongation at 72°C for 5 min. For the rs1256049, the PCR reaction program was as follows: 5 minutes at 95°C, 35 cycles of 95°C for 50 s, 54.5°C for 50 s, 72°C for 50 s, and final elongation at 72°C for 5 min. The rs4986938 polymorphism (which results in a band size of 646 bp for the normal estrogen receptorβ sequences (GG), a total of three distinct bands of 646, 445, and 201bp in a heterozygous one (GA), and two distinct bands of 445 and 201 bp in the homozygous polymorphism (AA) were produced when the PCR products were digested using the AluI restriction enzyme (Fig. 1).

Bands of sizes 582 bp were produced in the normal ERβ sequence (GG) by restriction digestion of the rs1256049 polymorphism with the RSAI enzyme; three distinct bands of 582, 293, and 289 bp were produced in the heterozygous polymorphism (GA); and two distinct band of 293 and 289 bp, respectively, in the homozygous variants (AA) (Fig. 2).

The amplification results were subjected to 1.5% agarose gel electrophoresis analysis.

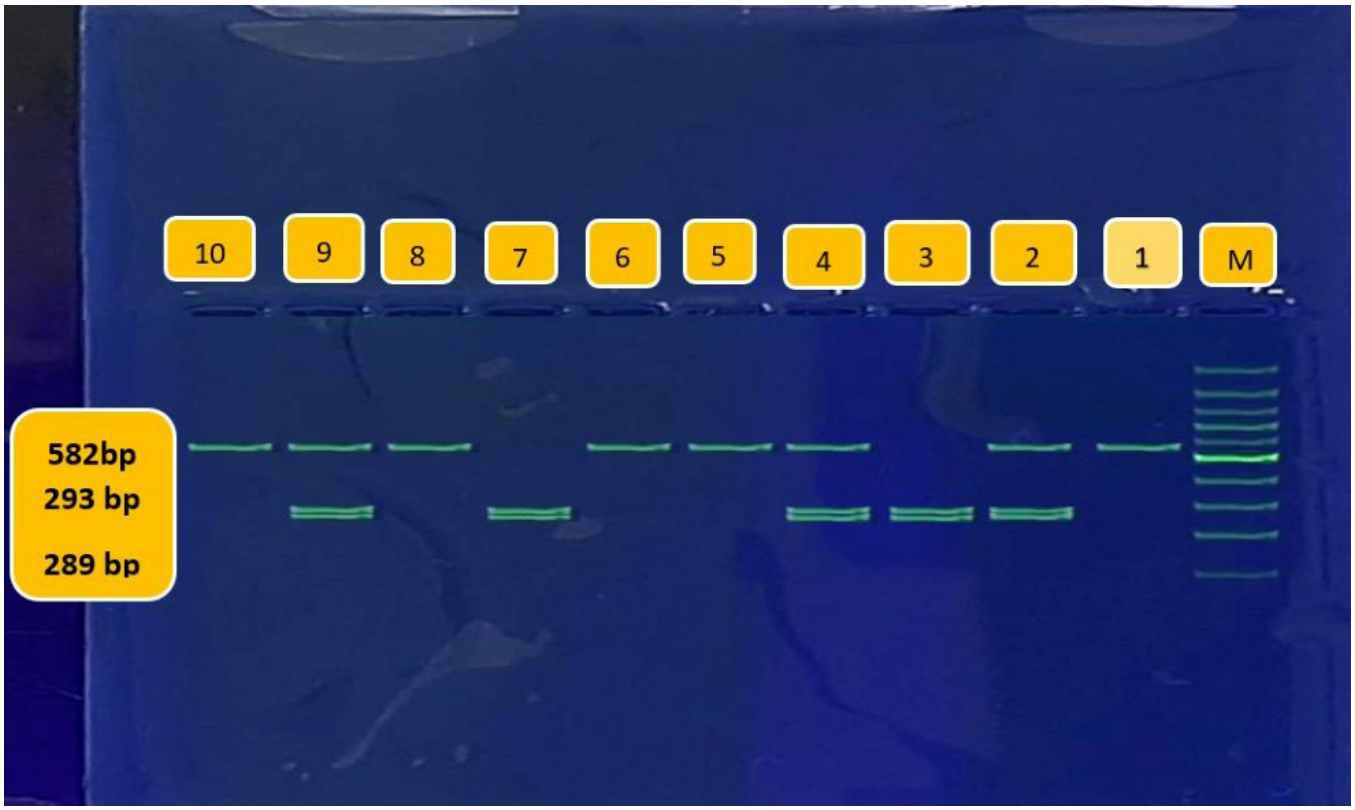

Fig. 1. Result of ERβ gene polymorphism (rs1256049) products on agarose gel electrophoresis following RSAI restriction enzyme digestion, visualization under UV light uses a green stars stain that indicated the presence of the allele. Lane M: ladder of 100 bp. Lanes 2, 4 and 9: (GA) genotype 582, 293 and 289 bp. Lanes 3 and 7: (AA) genotype 293 and 289 bp. Lanes 1, 5, 6, 8 and 10: (GG) genotype 582.

STATISTICAL ANALYSIS

ANOVA and the student t-test were utilized to compare patient's and control groups' continuous data. The Chi-square test was used to determine if there were any significant differences between the CAD and the control groups' genotype distributions and allele frequency.

RESULTS

The research groups' characteristics are listed in Table 1. Anthropometric parameters showed a difference between the CAD and control groups. When comparing the illness group to the control group, there was a substantial increase in atherogenic blood lipid concentrations and a decrease in HDL-c. Furthermore, the patient's group had significantly higher plasma atherogenic index or triglyceride/HDL-C ratios than the healthy ones. When comparing the GA carriers in the CAD group to the control group, the genotyping of thers4986938 G\ASNP in estrogen receptorβ revealed significant (OR =2.79, CI 95%=1.62 - 4.79, P =0.00) increases compared with the controls group (Table 2), for the GA carriers of the CAD group. The genotype analysis of thers1256049 SNP failed to exhibit a significant variation between patient and control group (Table 3). Atherogenic index and serum lipid concentration TC, TG, VLDL-C, LDL-C, HDL-C markedly elevated with the rs4986938 G\A polymorphism and TC, TG, VLDL-C, HDL-C (not for LDL-C) have been elevated with the rs1256049 G\A polymorphism (Tables 4-5).

DISCUSSION

Coronary artery disease (CHD) is a complicated medical condition that can be affected by hereditary and environmental factors [29]. Research on the role of many genes in the development of CHD is ongoing to understand its origins, alleviate treatment approaches, and understand its progression. This research focuses on the association between ERβ gene variation and cardiovascular disease in the Iraqi population, the first of its kind in humans. The study examined the association between variations in the ERβ gene (rs1256049 and rs4986938 SNPs) with CHD in the Iraqi population. These SNPs were chosen due to their known associations with cardiovascular characteristics and frequency-based confirmation of these associations [20-21]. The study found that the distribution of genotypes in the Iraqi population was stable from generation to generation, as the investigated SNPs were in obvious harmony with the HWE in the control group. Significant increases in atherogenic lipids were seen in the

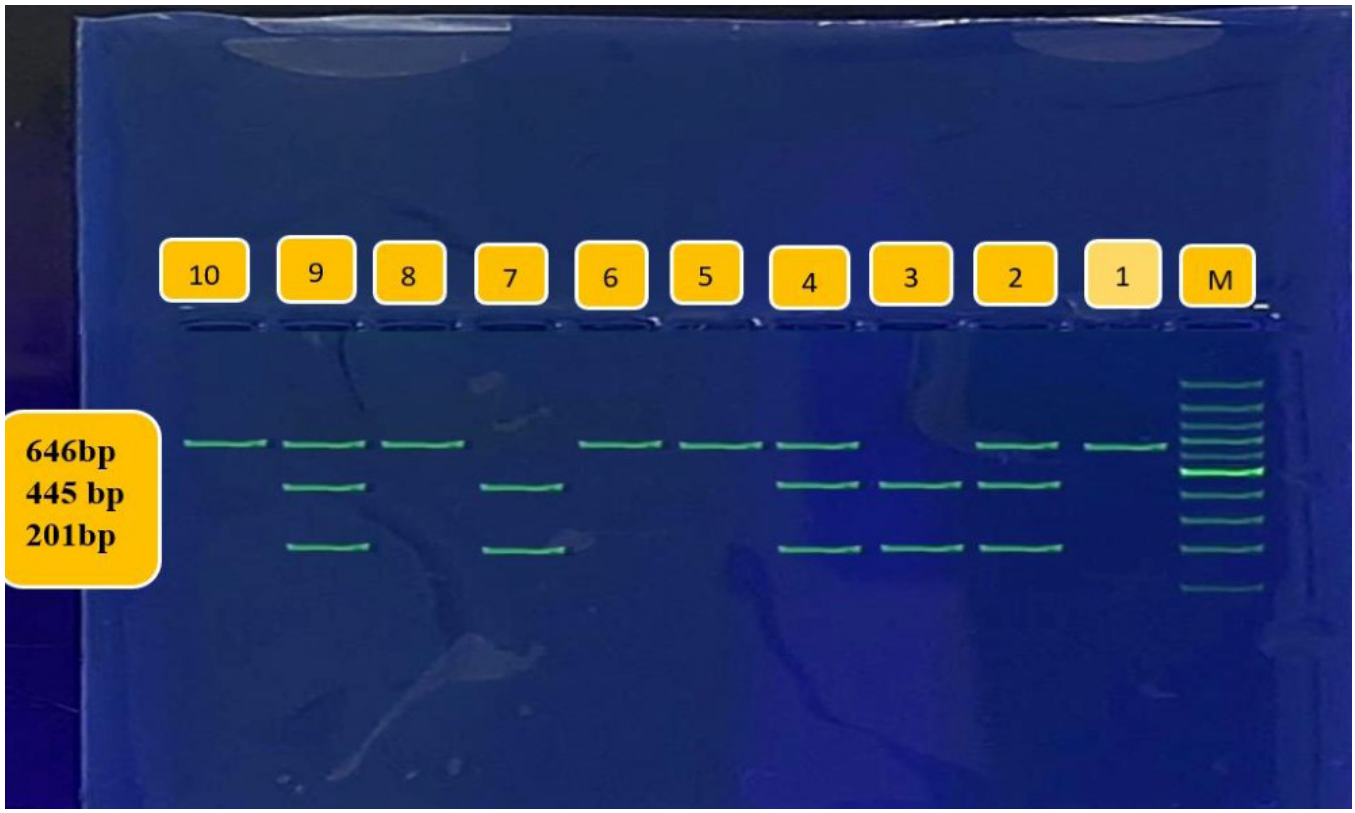

Fig. 2. ERβ polymorphism (rs4986938) results on electrophoresis of agarose gel following ALUI enzyme digestion. Direct detection under UV light using a green star stain that indicated a presence of alleles allowed for confirmation. The ladder of DNA 100 bp in Lane M. Lane1, 5, 6, 8&10: genotype (GG) 646 bp. Lane 2, 4 &9: (AG) genotype 646, 445 and 201bp.Lanes 3 and 7: (AA) genotype 445 and 201bp.

serum of both female and male CHD patients when compared to a control group. Atherogenic dyslipidemia is a major risk factor for the onset of atherosclerosis. LDL-c assessment alone, while looking for cardiovascular risk, would overlook the TG-rich lipoproteins. Our findings corroborate those of others [22-23] in showing that both the atherogenic index of plasma (AIP) and the atherogenic index (AI) are significantly higher in patients than in controls. The study reveals significant changes in remnant cholesterol (RC) and a high risk of coronary heart disease (CHD) in patients with advanced atherosclerosis (CAD). The atherogenic index in plasma (AIP) was higher in CAD patients than in the control group, indicating a greater CAD risk. ER signaling and ER gene polymorphism contribute to CAD etiology, with ERβ being more common in blood vessel tissue [24]. Recent research has shown that estrogen acting through ERβ may have anti-fibrotic effects on the heart [25-26]. Overexpression of the ERβ gene has been linked to improved cardiac function and survival in patients with a history of recent MI [27]. Vasodilation and the increase of eNOS expression are two further possible cardioprotective mechanisms of the ERβ gene in ischemia/reperfusion (I/R) damage, notably in women [28]. Coronary calcification is a hallmark of advanced atherosclerosis, and several studies have shown that ERβ is the most common estrogen receptors in human coronary arteries. Atherosclerosis and calcification progress in patients with elevated Erβexpression, hypertension and other cardiovascular illnesses [29-30], the link between ESR βpolymorphisms and LV structural variations [31-32], and so on. Genotypic analysis of the examined SNPs revealed that the rs1256049 G>A SNP is not associated with increased risk of coronary heart disease (CHD) in three different inheritance patterns: co-dominant, dominant, and recessive. These results suggest that rs1256049 in the ER βgene may have not a role in the onset of CHD in individuals from Iraq. Further investigation into the molecular basis is warranted. The single-nucleotide polymorphism rs 1256049 (or G1082A) are present in exon5, and it may alter ER2 gene expression and protein levels. Studies have linked rs 1256049 to elevated plasma lipoprotein concentrations and coronary artery disease in linkage disequilibrium with other loci [33]. In a case-control study of Chinese Han women, no evidence was found linking the genotypes or haplotypes of these two SNPs to an increased risk of coronary artery disease [34]. However, patients with the AG genotype of rs4986938 had a substantially decreased risk of CAD compared to those with the GG genotype, even among those younger than 40 years of age. The risk of coronary artery disease was greater

Table 1. Anthropometric and biochemical factors levels of CAD and control groups

Parameters	Control		CAD		*P*-Value
	Mean	SD	Mean	SD	
Age (y)	53.25	8.83	52.06	8.88	0.24
BMI (kg/m^2)	28.22	3.07	26.52	2.70	<0.0001
TC(mg/dl)	284.41	102.89	189.61	89.80	<0.0001
TG(mg/dl)	284.86	76.71	160.85	82.89	<0.0001
VLDL-C (mg/dl)	56.97	15.34	32.04	16.69	<0.0001
LDL-C(mg/dl)	193.17	99.14	115.59	88.52	<0.0001
HDL-C(mg/dl)	34.27	13.10	41.42	17.02	<0.0001
TG/HDL-C	0.93	0.27	0.58	0.36	<0.0001
LDL/HDL	6.98	4.80	4.26	4.98	<0.0001
CRI (TC/HDL)	9.95	5.64	6.38	5.94	<0.0001

Table 2. Differences in allele frequencies and genotype results of rs4986938 (AluI) SNP between patients and controls

	Patient (N=150)		Control (N=150)		OR (95%CI)	*P*-value
	No.	[%]	No.	[%]		
GG	90	60.0	121.0	80.7		
GA	54	36.0	26	17.3	2.79 (1.62 - 4.79)	0.000
AA	6	4.0	3	2.0	2.68 (0.65 – 11.04)	0.15

Table 3. Differences in genotype results of rs1256049 (RsaI) SNP between CAD and control groups

	Patient (N=150)		Control (N=150)		OR (95%CI)	*P*-value
	No.	[%]	No.	[%]		
GG	104	69.3	113	75.3		
GA	41	27.3	33	22.0	1.34 (0.79 - 2.29)	0.26
AA	5	3.3	4	2.7	0.86 (0.22 – 3.32)	0.65

Table 4. Results of phenotypic parameters of CAD patients analyzed in relevance to the rs4986938 (AluI) gene

	GG (N=90)		GA (N=54)		AA (N = 6)		*P*-value
	Mean	SD	Mean	SD	Mean	SD	
Age (y)	53.42	9.13	52.67	8.60	55.83	6.62	0.67
BMI (kg/m^2)	27.01	2.71	30.02	2.65	30.17	3.19	0.000
TC (mg/dl)	258.66	86.37	323.81	114.97	316.17	112.62	0.001
TG (mg/dl)	261.32	82.36	320.35	50.00	318.50	56.29	0.000
VLDL-C (mg/dl)	52.26	16.47	64.07	10.00	63.70	11.26	0.000
LDL-C (mg/dl)	167.98	83.87	231.82	109.45	223.30	109.83	0.001
HDL-C (mg/dl)	38.42	14.52	27.93	6.97	29.17	8.40	0.000
TG/HDL-C	0.84	0.30	1.07	0.15	1.05	0.17	0.000
LDL/HDL	5.66	4.42	8.96	4.69	8.65	5.36	0.000
CRI (TC/HDL)	8.33	5.29	12.42	5.27	12.03	6.12	0.000

among AA homozygotes. In another Iranian investigation, rs1256049 SNP showed no such associations, while rs1256049 genotypes were shown to be significantly associated with CAD [35]. Variation in starting conditions is mostly responsible for discrepancies in results. A study of phenotypic data stratified by genotype (GG, AG, and AA) for the rs 1256049 polymorphism found that total cholesterol, very low-density lipoprotein (VLDL), and triglyceride levels were considerably higher in the three genotypes. In this study, has been found that the ER rs4986938 SNP was found to elevate the risk of coronary heart disease in the GA genotype with (OR= 2.79, CI(1.62-4.79), P=0.000). The link between rs4986938 polymorphisms and the risk of cardiovascu-

Table 5. Results of phenotypic parameters of CAD patients analyzed in relevance to the rs1256049 (Rsal) gene

	GG (N=93)		GA (N=50)		AA (N = 7)		P-value
	Mean	SD	Mean	SD	Mean	SD	
Age (y)	53.69	8.91	52.07	8.67	53.60	9.40	0.61
BMI (kg/m2)	27.43	2.97	30.05	2.52	29.60	2.88	0.000
TC (mg/dl)	270.12	98.82	313.83	101.65	340.60	146.50	0.031
TG (mg/dl)	268.47	80.82	323.98	49.12	305.00	60.80	0.000
VLDL-C (mg/dl)	53.69	16.16	64.80	9.82	61.00	12.16	0.000
LDL-C (mg/dl)	179.74	95.80	220.55	97.29	248.00	137.94	0.36
HDL-C (mg/dl)	36.68	14.36	28.49	7.44	31.60	5.37	0.002
TG/HDL-C	0.87	0.29	1.07	0.15	0.98	0.14	0.000
LDL/HDL	6.34	4.90	8.40	4.21	8.39	5.42	0.53
CRI (TC/HDL)	9.13	5.78	11.86	4.80	11.39	6.04	0.26

lar disease (CVD) occurrence and severity is not without controversy, while one study conducted on the American population confirmed no association between the A allele of rs 4986938 SNP, another case-control study conducted on the Polish population suggests that the ESR2 rs4986938 (G1730A) variant may play a role in the risk factor of MI at a young age, albeit not as an independent but as a potential risk, ESR βgene polymorphism AluI (G1730A) was shown to be correlated with male body fat percentage in a study of 170 healthy Greeks (ages 22-59, mean 42 years) [36]. Researchers believe that ethnic variations among the studied populations account for the conflicting findings in the literature regarding the connection of certain ESRβ genotypes with BMI, body fat mass, hypertension, LDL, HDL, triglyceride, and glucose concentrations. The population we studied was composed entirely of Caucasian Poles. Despite the prevalence of young-age MI in males, there is currently little information available on the association between premature CAD and ESRβ genotypes in this cohort. Coronary artery intima from asymptomatic males who had atherosclerotic plaque on autopsy was shown to have elevated ER expression [37]. Plaque area associated favorably with ER expression, indicating a function for ER in both advanced atherosclerosis and early CAD. There was a correlation between the G1730A mutation and LDL level [38] in healthy young males. In conclusion, by studying the association between ER βgene variation and CHD, researchers can identify individuals at high risk for developing CHD and those who may be genetically protected against the illness. Further research is needed to fully understand the complex relationship between genetic predisposition and environmental factors in the development of CHD.

LIMITATIONS

1. Conduct a study with a large enough sample size to allow for separate men and women analyses.
2. Analysis of numerous SNPs of ERβ gene and analyzing their relation with other genes to discover which one is more prevalent in our community.
3. Assessment of gene-gene interaction of ERβ gene with other CHD relation genes.

CONCLUSION

The rs4986938 (not for rs1256049SNPs) are risk factors to develop CHD in Iraqi population and implicated in changes of serum lipid concentrations and atherogenic index.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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