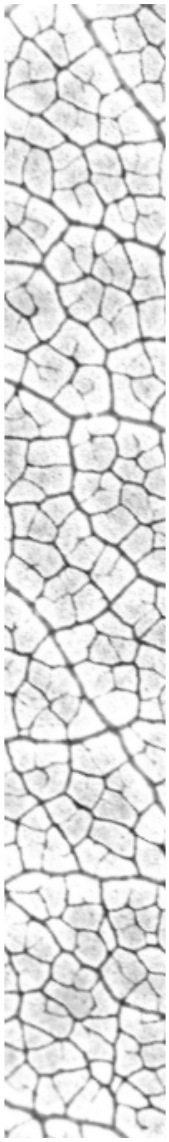

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Cholesteryl ester transfer protein Taq1B gene polymorphism in some Iraqi patients with lipid disorders

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Abstract
Cholesteryl ester transfer protein (CETP) Taq 1 B polymorphism (rs708272) effect on CETP concentration that has a major role in the lipoproteins metabolism and subsequently lipid profile. Therefore, this study aimed to assess the association between CETP Taq1B polymorphism and lipid disorders among Iraqi patients. Hundred and thirty subjects, 80 patients with lipid disorders and 50 healthy age ranged from 25 to 60 years were recruited by private clinic from Iraqi population. Blood samples were collected and used to separate serum that was used to measure lipid concentrations by enzymatic assays and to extract the DNA that was used to detect CETP Taq1B polymorphism using polymerase chain reaction restriction fragment length polymorphism (PCR-RELP). Results showed statistically increase ($p < 0.01$) in lipid profile concentrations and highly significant differences ($p < 0.01$) of alleles and genotypes frequency in patients group compared to controls. Patients with B1B1genotype showed a higher high-density lipoprotein cholesterol (HDL-C), triglyceride (TG) and total cholesterol (TC) levels but not significantly and statistically increase ($p < 0.01$) in low lipoprotein density cholesterol (LDL-C) levels compared with B1B2 and B2B2. We suggest that CETP Taq1 B polymorphism was significant association with Iraqi patients with lipid disorders compared to controls. The genotype B1B1 and B1 allele can be considered as a marker of genetic predisposition to lower HDL-C levels and higher TC and TG levels in the Iraqi population and lead to increased susceptibility of the cardiovascular disease.

Keywords: Taq1B gene polymorphism, lipid disorders

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INTRODUCTION

Serum lipid abnormalities are recognized as major risk factors for modifiable cardiovascular diseases and were identified as independent risk factors for essential hypertension (Osuji et al. 2012). The lipid profile test, including total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoproteins cholesterol (VLDL-C), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) have a high predictor for cardiovascular incidence in adulthood, therefore, the American Heart Association recommended that the lipid profile must be periodically evaluated (Juliaty et al. 2015). Genetic diagnosis using new molecular biology techniques enable the mechanisms underlying the individual and family predisposition to certain diseases to be studied (Ortega et al. 2002). Gene polymorphisms are markers of biological diversity and certain genotypic variations relate to specific human disease phenotypes (Alharbi et al. 2013). In addition, a single nucloetide polymorphism (SNP) can play a limited role in the overall genetic load for the disease and both common and rare gene polymorphisms can influence the susceptibility to the disease differently (Licastro et al. 2010). Understanding the effects of environmental, genetic and lipid levels in particular on the state of health is of great interest (Clifford et al. 2013).

Cholesterol transmission protein (CETP) is a hydrophobic glycoprotein that plays a major role in the metabolic regulation of cholesterol. It is expressed in many tissues, including the liver, spleen, adipose tissue, small intestine, kidney, heart and skeletal muscles (Iwanicka et al. 2018). CETP is a single gene contains 25 kilo base genomic DNA and was located on the long arm of chromosome 16, it consists of 16 exons and 15 introns (Hassanzadeh et al. 2009). Genetic changes of CETP can lead to modification in the concentration and function of CETP that can cause a change in the level of LDL-C and HDL-C. These CETP gene polymorphisms include I405 V, -629C/A, Taq1B and D442G can cause disease due to the change in the serum lipids pattern (Akbarzadeh et al. 2012). Taq1B rs708272 (5454G>A) is a polymorphism in the first CETP intron at 279th

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nucleotide and has a restriction site for Taq1 endonuclease. Taq1B polymorphism may affect the plasma CETP activity and its concentrations as well as HDL-C levels (Heidari-Beni et al. 2015).

Taq1B polymorphism was the first genetic variation related to HDL- C plasma levels. The less common B2 allele (absence of the TaqI restriction site) may be linked to a lower CETP mass and a higher HDL-C level than the more common B1 allele (presence of the restriction site for enzyme Taq1) (Galati et al. 2014). The causes of differences and similarities observed in many Taq1B polymorphism studies may not be clear due to different reasons, such as sample size differences, environmental factors, ethnic factors, patient selection criteria and control groups, lifestyle and diet (Maroufi et al. 2016).

Genetic variation in the CETP gene especially Taq1B polymorphism has been extensively studied in various populations for association with variation in lipid profile (Garcia-Rios et al. 2018). Therefore, the study of this polymorphism in Iraqi patients may be of interest because of the genetic diversity of populations. Thus, this study aimed to assess whether CETP Taq1B polymorphism as a risk factor of the lipid disorders and clarify effect of this polymorphism on lipid profile for some patients with lipid disorders in Iraqi population.

MATERIALS AND METHODS

Subjects Selection

A total number of the study sample was 130 subjects, 80 individuals were patients with lipid disorders and 50 individuals were healthy have been selected as a control, age ranged from 25 to 60 years and sex were matched in both groups, volunteers were recruited by private clinic from Iraqi population.

Sample Collection

At the time of clinical examination, 5 ml of blood samples were collected from each subject and divided into two parts: In the first part 1 ml of blood has been collected, while in the second part 4 ml was taken in a normal test for separation of the serum.

Laboratory Measurements

Serum lipid profile TC, HDL-C and TG levels were measured by enzymatic colorimetric methods (Naito et al. 1984a, 1984b), and the LDL and VLDL was estimated by Friedewald formula (Friedewald et al. 1972).

Determination of CETP Taq1B Gene Polymorphism

Extraction of genomic DNA was carried out by using (Ali et al. 2008) method. The purity and integrity of genomic DNA was measured by the absorbance ratio of 260 nm to 280 nm (A260/A280) and the high molecular weight and good quality of the electrophoresis of agarose gel respectively. Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) technique was used to determine the genotype of the CETP Taq1 B gene according to (Ahmed et al. 2011). The following primers forward 5-CAC TAG CCC AGA GAG AGG AGT GCC-3 and reverse 5-CTG AGC CCA GCC GCA CAC TAA C-3 were used to amplify a fragment of 535 bp from intron 1 of the CETP gene. PCR amplification was performed in a total volume of 20 µL including 10 µL of 2X Go Taq green master mix supplied by Promega company (USA), 4 µL of genomic DNA, 1 µL of each primer in addition to 4 µL of DNase/RNase free water. PCR cycling was carried out according to the following program: one cycle for 5 minutes at 95 °C followed by 30 cycles, each cycle includes 95 °C for 30 seconds, 63 °C for 30 seconds and 72 °C for 45 seconds with one cycle of 5 minutes at 72 °C for a final extension. The resulting of PCR product (535 bp) was determined by using a 2% agarose gel electrophoresis stained with red safe. Ten units of the Taq I restriction enzyme (New England, BioLabs, Inc.) was added to 5 µl of the PCR product and incubated at 65 °C for 1 hour. The digest fragments were visualized on 2% agarose gel electrophoresis stained with red safe in the presence of 100 bp DNA ladder (Biolabs-England). Three types of bands were shown, single fragment (535 bp) as a B2B2 homozygous indicate the absence of the TaqI restriction site, 2 fragments (361 and 174 bp) as a B1B1 homozygous indicate the presence of the restriction site and 3 fragments (535, 361, 174 bp) as the B1B2 heterozygous.

Statistical Analysis

SPSS version 20 PC programming were utilized to statistical analyses. Hardy– Weinberg equilibrium and recurrence of alleles and genotypes in addition of odds ratios (OR) and their 95% confidence intervals (CI) of the patients and control group were determined utilizing Pearson's chi-square test; $P<0.05$ was viewed as significant and $P<0.05$ as high significant. One-way ANOVA and student's t-test were utilized for the comparison of mean ± standard deviation (SD) of lipid parameters between healthy and patients group and among the genotypes of CETP Taq1 B polymorphism.

RESULTS AND DISCUSSION

Serum Lipid Results of the Population Study

In this study 130 Iraqi subject were enrolled, 80 patients with lipid disorders and 50 as a control. **Table 1** shows serum lipid results of the population study, (TC, TG, LDL-C, and VLDL-C) were significantly higher ($P<0.0001$) except HDL-C was lower in the patient group compared to the control group ($P<0.0001$). The resulted data reveal to that patients may have susceptibility to atherosclerosis.

Table 1. Comparison between lipid profiles of the population study

Parameters	Patient group (No. 80)	Control group (No. 50)	*p* value
HDL-C (mg/dL)	41.7 ± 6.72	48.6 ± 5.23	0.0001**
TC (mg/dL)	177.0 ± 7.8	114. ± 9.3	0.0001**
LDL-C (mg/dL)	89.5 ± 7.9	68.3 ± 6.33	0.0001**
VLDL-C (mg/dL)	28.1 ± 3.08	18.8 ± 3.44	0.0001**
TG (mg/dL)	138.81 ± 5.0	90.0 ± 2.9	0.0001**

** significant P ≤ 0.01

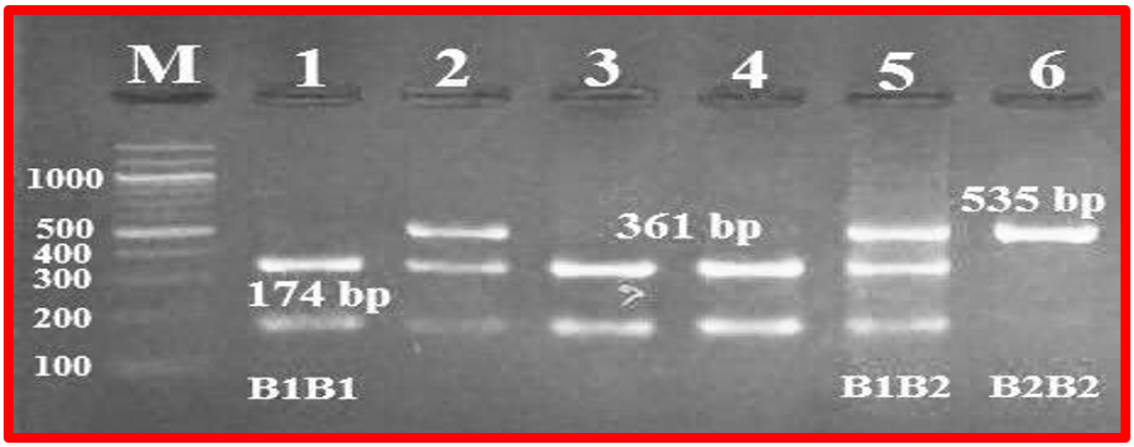

Fig. 1. Represents 2% agarose gel electrophoresis of the PCR-RFLP product showing genotypes of CETP Taq1 B gene polymorphism: lane (M) 100 bpDNA ladder, lane (1,3 & 4) B1B1 homozygote (361 & 174 bands), lane (5) B1B2 heterozygote (535, 361 & 174 bp bands), lane (6) B2B2 homozygote (535 bp band)

Table 2. Distribution of genotypes and alleles frequency of the patients and controls group

Genotypes	Patients No. (80)		Control No. (50)		p value	OR	(95% CI)
	No.	%	No.	%			
B1B1	30	37.5	6	12		6.15	1.96 - 19.28
B1B2	37	46.25	28	56	0.0036**	1.63	0.67 - 3.93
B2B2	13	16.25	16	32		1 Ref.	-
Alleles	**No.**	**%**	**No.**	**%**	**p value**		
B1	97	60.62	40	40		2.3095	1.386 - 3.848
B2	63	39.38	60	60	0.001**	1 Ref.	-

** significant P ≤ 0.01

Genotypes and Alleles Frequency

By PCR-RFLP analysis of the CETP Taq1 B polymorphism, three types of genotypes (B1B1, B1B2, B2B2) have been obtained as show in (**Fig. 1**).

Table 2 shows results of the genotype and allelic frequencies in (%) and number of patients having each genotype of study population. The distribution of genotype in case and control group was conducted in the Hardy-Weinberg equilibrium. The results show that there is a significant differences (p value 0.0036) between frequency of genotypes and alleles of CETP Taq1 B polymorphism in the patient and control groups. Patients with B2B2 genotype (16.25%) were significantly lower while both the B1B1 (37.5%) and B1B2 (46.25%) genotypes were higher compared with the control. Also we observed that there is an increasing in the B1 allele frequency on the contrary B2 allele in the patient compared to the control group (p value 0.001).

Patients had B1B1 genotype (OR 6.15 and 95% CI 1.96 - 19.28) and high value of OR of B1 allele (2.3095) with (95% CI 1.386 - 3.848), according to these results B1B1 genotype may be considered as a risk factor while B2B2 genotype and B2 allele seem to be protective for lipid disorders in Iraqi population.

Effect of CETP Taq1 B Polymorphism on Serum Lipid Profile of the Population Study

The effect of CETP Taq1 B polymorphism on lipid profiles in the Iraqi population is shown in **Table 3**. In the patients group, there are no significant differences between the HDL-C levels of B1B1, B1B2 and B2B2 subjects, although the B2B2 individuals have mean HDL-C levels (41.78 ± 6.91) compared to other groups.

Mean value of HDL-C level (40.86 ± 6.61) of patients with a B1B1 genotype (No. 13) had approximately similar to the HDL-C mean value (40.84 ± 6.87) of the patients with B2B2 genotype (No. 30). This indicates that the presence of allele B2 may be associated with an increasing concentration of HDL-C which plays a role in protection against lipid disorder. Also the results in **Table 3** show that there was non-significant increase in levels of TC and TG except VLDL-C in individuals with B1B1 genotype than in other genotypes while patients with B1B1 genotype have significant increase (p value 0.0009) in the LDL-C compared to other genotypes.

Table 3. Serum lipid levels of the population study according to CETP Taq1 B polymorphism

Patients No. 80				
Parameter	**B1B1 No. = 30**	**B1B2 No. = 37**	**B2B2 No. = 13**	**P value**
HDL-C (mg/dL)	40.86 ± 6.61	41.78 ± 6.91	40.84 ± 6.87	0.893
TC (mg/dL)	182.16 ± 5.46	179 ± 9.48	161.38 ± 3.46	0.06
LDL-C (mg/dL)	96.23 ± 2.75	91.24 ± 2.10	69.15 ± 2.64	0.0009**
VLDL-C (mg/dL)	27.63 ± 7.16	27.56 ± 6.26	30.69 ± 7.10	0.536
TG (mg/dL)	140.4 ± 3.45	139.83 ± 3.08	132.23 ± 5.09	0.719
Controls No. 50				
Parameter	**B1B1 No. = 6**	**B1B2 No. = 28**	**B2B2 No. = 16**	**P value**
HDL-C (mg/dL)	48.16 ± 6.91	48.64 ± 4.53	48.81 ± 6.02	0.968
TC (mg/dL)	121.16 ± 9.09	111.17 ± 9.66	117.31 ± 8.89	0.399
LDL-C (mg/dL)	66.66 ± 4.32	69.67 ± 7.62	66.56 ± 3.48	0.234
VLDL-C (mg/dL)	16.83 ± 2.63	19.75 ± 3.54	18.0 ± 3.11	0.081
TG (mg/dL)	84.16 ± 16	98.82 ± 7.75	90.0 ± 5.59	0.079

**** significant P ≤ 0.01**

DISCUSSION

A number of single nucleotide polymorphisms have been described in the CETP gene. It has been reported that the rs708272 Taq1 B (g.5454G>A) polymorphism influences HDL-C concentration. Therefore, the aim of this examination was to assess the polymorphism of CETP gene at Taq1B site and its effect on serum lipid in Iraqi subjects with lipid disorders. We selected CETP Taq1 B polymorphism since a few examinations that discussed its relationship to the HDL level and in light of the fact that we didn't find any Iraqi investigation talking about this polymorphism of the CETP gene.

Results in **Table 1** shows high significant differences (P<0.01) of serum lipid (TC, TG, LDL-C, and VLDL-C) except HDL-C was lower in the patient group compared to the control group (P<0.01). High serum concentrations of TC, TG, LDL, and diminished HDL are known to be related with significant hazard factors for CVD. Examples of lipid irregularities among Asians and their relative effect on cardiovascular hazard haven't very much described (Karthikeyan et al. 2009). Low HDL is progressively perceived as a free risk factor for unfavorable CVD results, regardless of concentrations of LDL. These information strongly propose that low HDL is a clinically huge issue (Bruckert et al. 2005).

The examination of frequency circulation of Taq1B polymorphism demonstrated a critical distinction between patients with lipid disorders and the healthy group. The B2B2 genotype was significantly higher (32 %) in the control group than in the patients group (16.25 %), showing a conceivable defensive job of this genotype. On the contrary, we note a high frequency of B1B1 genotype (37.5 %) in the patients group compared with the healthy group (12 %). This can indicate the possibility of this genotype being associated with the genetic predisposition of the lipid disorder, especially that the frequency of allele B1 was (60.62 %) and the odds ratio value (2.3095) of the patients compared to frequency (40 %) in the healthy group. These results were disagreed with Ahmed AI et al, 2011(18) whose found some differences in the frequency of alleles and genotypes between patients and healthy groups but not significant, this may because the small sample size used. CEPT establishes the key component of cholesterol turn around exchange – the defensive framework against the improvement of atherosclerosis. Based on a few studies, it is conceivable to express that polymorphism Taq1B of the CETP gene is the determinant of the CEPT level (21).

In addition, when we assessed the effect of polymorphism Tag1 B of gene CETP on the concentration of serum lipid profile in the study population, we found that the similar HDL-C levels in patients (B1B1 40.86 ± 6.61, B1B2 41.78 ± 6.91 and B2B2 40.84 ± 6.87) and control (B1B1 48.16 ± 6.91, B1B2 48.64 ± 4.53 and B2B2 48.81 ± 6.02) group, this can be attributed to the lifestyle and the environment of the participants and in addition to their low number in the study which may debilitate the statistical intensity of potential connections between CETP Taq1 B polymorphism and the serum lipid parameters. On the other hand, in the patients group, the presence of the B1B1 genotype was found to be associated with high TC and TG levels although not statistically significant (P<0. 05), so, B1B1 genotype may be considered as a risk factor for lipid disorder in Iraqi society. The results of this study were consistent with the findings of Yilmaz H, et al, 2004 (22-25) indicating the high level of TG levels with the B1B1 genotype of the group of type 2 diabetes patients in Turkish society.

In conclusion, our results indicate that CETP Taq1 B polymorphism was significant association with Iraqi patients with lipid disorders compared to controls. The genotype B1B1 and B1 allele can be considered as an marker of genetic predisposition to lower HDL-C levels and higher TC and TG levels in the Iraqi population and lead to increased susceptibility of the cardiovascular disease. Other studies should be conducted using a larger number of samples in different locations of Iraq to confirm these results.

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