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Assessment of Cholesteryl Ester Transfer Protein Gene Taq1B Polymorphism and Its Relationship with Lipid Levels of Hypertensive Iraqi Patients

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ABSTRACT

Lipid disorder is one of the main causes of essential hypertension. The polymorphism of cholesteryl ester transfer protein (CETP) gene was well-known to be related with the variants in the lipid profiles. Therefore, the study was targeted to determine the relationship between the polymorphism of CETP Taq1 B (rs708272) and evaluate its relationship with lipid profile levels among some hypertensive Iraqi patients. One hundred and seventy blood samples were collected from two groups, the first group included a hundred hypertensive patients and the second group included seventy healthy individuals as control group. For both groups, lipid profile was estimated, genomic DNA was extracted from all samples and Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) technique was used to detect CETP gene Taq1 B polymorphism. Results indicated that the concentrations of triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL-C), and very-low-density lipoprotein (VLDL-C) had increased, whereas the concentration of high-density lipoprotein (HDL-C) was decreased in hypertensive patients in comparison to control group. The study also showed a higher Frequency for the genotype B1B1 and the (B1) allele in hypertensive patients in compare with control group ($p \leq 0.01$). Lipid profile concentrations according to CETP gene Taq1 B genotypes showed non-significant differences. The conclusion of this study was indicated that the polymorphism of CETP gene Taq1B may be related with lipid disorder and CETP (B1) allele which could be used as a genetic marker for increasing hypertensive susceptibility in Iraqi population.

Keywords- Hypertension, Lipid disorders, Taq1B gene polymorphism.

I. INTRODUCTION

Hypertension is one of the most public disease in the world, especially in developing countries (1). Increased prevalence of hypertension is due to several factors such as obesity, smoking, sleeping disorder, dyslipidemia and increased intake of alcohol, caffeine and sodium (2). Hypertension is common in 50% of persons between 60-65 years and it is increased beyond seventy years (3). One of the main causes for essential hypertension is dyslipidemia which is a metabolic disorder of lipoproteins secretion. Dyslipidemia more common in hypertensive patients and may be affects by both genetic and environmental factors (4, 5). Hypertension was known associated with variations in lipid metabolism, therefore, hyperlipidemia could detected in hypertensive patients (6). Hypertension widely associated with cardiovascular diseases and increased serum concentrations of the low-density lipoprotein-cholesterol (LDL-C), triglyceride (TG), and total cholesterol (TC). While the little concentration of the high-density lipoprotein-cholesterol (HDL-C) was considered as the central risk factor for death (7). Several studies have shown that (TC), (TG) and all lipoproteins were abnormal among hypertensive patients compared with healthy individuals (8, 9). The relationship between hypertension and HDL-C levels with coronary heart disease discussed in a study showed that the HDL plays a critical role in the safeguard of blood vessels from atherosclerosis (10).

The major protein that regulates the reverse transfer of cholesterol from the peripheral tissues to the liver is Cholesteryl ester transfer protein CETP (11), where it works to stimulate the alternation of cholesteryl ester from HDL-C to LDL-C and VLDL-C in place of triglycerides (12). The important role of CETP in the metabolism of lipoprotein was proved by the high levels of HDL-C in patients with genetic CETP deficiency (13). The main production site of CETP protein is in the liver, adipose tissue, and spleen, while minimized levels of CETP protein produced in the kidney, small intestine, heart, skeletal muscles, and adrenal gland (14). The activity of CETP can be modulated autonomously by CETP mass diversity, metabolic state, and in particular by TG- rich lipoprotein levels (15).

Genetic markers are connected with specific disease phenotype, they would be most useful as diagnostic tools of prognosis or response to therapy (16). The locus of human CETP gene in 16q 12-21 contains 25 kilo base pair, genomic and composed of sixteen exons and fifteen intron encoding (476) amino acids, the size of the exon ranges from 32 to 250 bp (17). CETP Genetic diversity is the main factor of inter-individual difference of the CETP levels (18). CETP gene contains several single nucleotide polymorphism (SNP) (19).

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Some of SNPs are related to CETP activity and other connected with HDL-C level (20). One of the common polymorphisms of the CETP gene is rs708272 Taq1 B that found in intron 1, which might affect the CETP activity, HDL-C levels, and the size of lipoprotein (18). The appearance of the Taq 1 restriction site was appointed by B1 and its absence by B2, the less common allele B2 was associated with decreased CETP activity and increased HDL-C level in compare to B1 allele (14, 21). Environmental factors such as obesity, smoking, alcohol intake and hypertriglyceridemia may affect the link between HDL-C level and CETP gene polymorphism (22).

For our knowledge, there were some studies to evaluated the association of CETP Taq 1B with hypertension such as (Schechter et al., 2010)(37) in United State America and (Niu et al., 2007)(28) in Chinese society. Also there were some researches to assessment the link between CETP and lipid profile like (Hassanzadeh et al., 2009)(17) in the Iranian society that verify the genotypes of CETP Taq 1B in the Iranian subjects with and without primary combined hyperlipidemia and (Kolovou et al., 2010) (36) in the Greece. While we did not find any study that included determining the polymorphism of CETP Taq 1B gene and its relationship with the concentrations of lipid profiles in the hypertensive patients of Iraqi population. Therefore, this study was aimed to evaluate the polymorphism of CETP Taq1B gene and its association with lipid concentrations in Iraqi hypertensive patients in Salah Al-din region.

II. MATERIALS AND METHODS

Research subjects: A total of 170 subjects were collected from Iraqi population, whose age is ranging from 25- 60 years. Out of them, 70 subjects as control group, the remaining 100 subjects were diagnosed as patients with hypertension. Gender was matched in both groups, and they were selected from private clinic.

Sample collection: For each subject, 5 ml of venous blood was collected and separated into two parts, the first has (1ml) was used for DNA extraction, and the second (4ml) was used to separate the serum.

Biochemical analysis: Serum TG, TC and HDL-C were determined by enzyme method according to (23, 24). Friedwald formula used to calculate LDL-C and VLDL-C (25).

Genotyping of CETP Taq1B polymorphism: Genomic DNA was extracted according to standard procedure (26). Absorbance at 260 and 280 nm was calculated using Nanodrop for determining concentration and purity of DNA samples, agarose gel electrophoresis at 0,8 % was used for checking the integrity of the DNA. The technique of PCR-RFLP has been used to detect the genotypes Taq1B polymorphism for the gene of CETP according to (27). The sequence of forward primer was 5-CAC TAG CCC AGA GAG AGG AGT GCC-3 and the sequence of reverse primer was 5-CTG AGC CCA GCC GCA CAC TAA C -3 which they were used to amplification the fragment of 535 bp from the intron number 1 of the CETP gene. The method of PCR was accomplished in (20 µl) having (10 µl) from 2X Go Taq green master mixture which was supplied by the Company of Promega (USA). Four microliter of the genomic DNA, (1 µl) of previous primer, and (4 µl) of the DNase/ RNase free water. The amplification protocol contains; an initial step of 5 min at 95 ºC, followed by 30 cycles of denaturation at 95 ºC for 30 sec, annealing 63 ºC for 30 sec and extension at 72 ºC for 45 sec with one cycle at 72 ºC for 5 min as a final extension. PCR product (535bp) was separated at agarose gel electrophoresis (2%) using red safe pigment. Taq 1 restriction enzyme (10 units) from Bio labs- New England. Inc., were appended to (5 µl) of PCR products then incubated at (65ºC) for one hour. Products of digestion were envisioned with the existence of (100 bp) of DNA ladder from Bio labs-England. There has been three types of bands appeared, a single fragment of (535 bp) for the homozygous B2B2 which point to the absence of the Taq1 restriction locate, and two fragments of (361 and 174 bp) for the homozygous B1B1 which point to the appearance of the restriction locate and the three fragments of (535, 361, 174 bp) for the heterozygous B1B2.

Statistical analysis: The statistical analysis has been used SPSS version 20 PC. Chi-square test of person was applied to determine the frequency of genotypes and alleles; as well as the ratios of odd (OR), and their confidence intervals (CI) 95% of control groups and patients, ($P< 0.05$) was considered significant and ($P< 0.01$) was highly significant. The student's t-test and ANOVA were utilized for the comparison of the lipid profile between patients and control groups and amongst the polymorphism of CETP Taq1B genotypes.

III. RESULTS

Lipid parameters: A total of 170 individuals were classified for two groups; seventy healthy persons as control group and a hundred hypertensive patients who are they 57 were males, and 43 were females. The table (1) showed that serum LDL-C levels and TG were higher significant ($p=0.0001$) in hypertensive patients (149.71±7.999, 197.877±8.825) when compared with control group (71.3±9.822, 74.7±7.033) respectively., the mean of TC level was significantly higher in the hypertensive patients (225.92±6.033) in compared with control group (160.814±11.185) ($p= 0.001$), and the mean of VLDL-C level was highly significant in patients group when comparison with the control group (41.43±7.286 vs. 32.285±5.538) ($p = 0.003$). However, there was a significantly low level of HDL-C in patients group when it compared to control group (45.91±2.040 vs. 51.7±8.377) ($p = 0.0006$). Table (2) showed there was no significant differences in the levels of serum lipids in the hypertensive patients according to sex.

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Table 1: Levels of lipid in the control and patients group.

Parameter	Patients (100)	Control (70) Mean ± SD	*P*. value
HDL-C (mg/dL)	45.91 ± 2.040	51.7 ± 8.377	0.0006 **
TC (mg/dL)	228.0 ± 6.1	160.814 ± 11.185	0.001 **
LDL-C (mg/dL)	153.0 ± 7.5	71.3 ± 9.822	0.0001 **
VLDL-C (mg/dL)	41.2 ± 7.6	32.285 ± 5.538	0.0003 **
TG (mg/dL)	198.0 ± 8.2	74.7 ± 7.033	0.0001 **

Table 2: Levels of serum lipid in the patients according to sex.

Parameter	Male (57)	Female (43) Mean ± SD	*P*. value
HDL-C (mg/dL)	46.754 ± 3.960	44.790 ± 8.921	0.422
TC (mg/dL)	228.087 ± 5.349	223.046 ± 8.451	0.679
LDL-C (mg/dL)	147.210 ± 4.259	153.023 ± 4.967	0.451
VLDL-C (mg/dL)	42.473 ± 5.645	40.046 ± 9.352	0.489
TG (mg/dL)	204.672 ± 8.238	189.186 ± 9.790	0.337

Genotypes and allele frequency: Genotyping of CETP Taq 1B polymorphism was detected by using RFLP-PCR technique. Results have shown that there was three patterns of genotypes B1B1, B1B2, and B2B2 in the following figure (1).

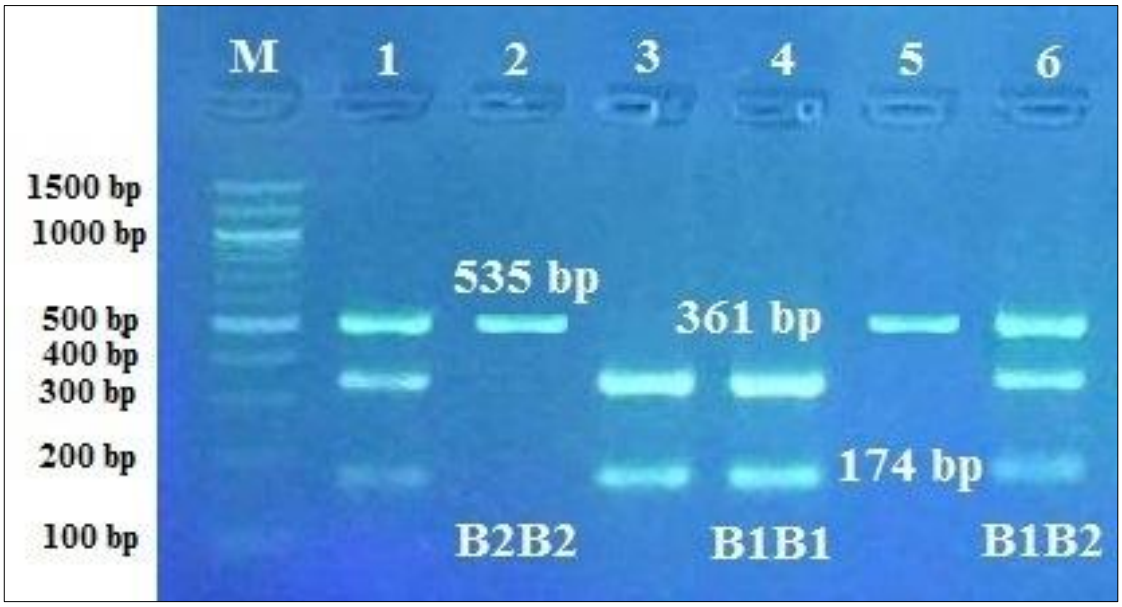

Figure 1: CETP Taq1 B gene polymorphism of PCR-RFLP products. Lane M: 100 bp of DNA ladder, the lane (3 & 4) of homozygote B1B1 (361, 174 bp) bands, the lane (1 & 6) of heterozygote B1B2 (535, 361, and 174 bp) bands, and the lane (2 & 5) of homozygote B2B2 (535 bp) band.

The CETP Taq 1B genotype and allelic frequencies for patients and control groups shown in table (3). The percentage of genotype recurrence of the B1B1: B1B2: B2B2 in patients and control are 44: 47: 9 and 8.57: 68.57: 22.86 respectively, while patients with B2B2 genotype are significantly lower (P = 0.0001) when compared with other genotypes. The frequency of the B1 allele was highly significant (P = 0.0001) when compared with B2 allele in the patients group. The analysis of odds ratio showed that B1B1 genotype has (13.04) and 95% CI (4- 42.47) and a high rate (2.769) of odds ratio for the B1 allele and 95% CI (1.771- 4.329) if compared by B2B2 genotype. Can be concluded from these results, that the allele B1 was related with an increased level of serum HDL-C.

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Table 3: The distribution of genotypes and the allele's recurrence of the control and patients group.

The genotypes	Patients (100)		Control (70)		*P*. value	OR	(95%) CI
	No	%	No	%			
B1B1	44	44	6	8.57	≤ 0.001*	13.04	4 - 42.47
B1B2	47	47	48	68.57		1.74	0.7 - 4.33
B2B2	9	9	16	22.86		1 Ref.	-
Alleles	No.	%	No.	%	*P* value		
B1	135	67.5	60	42.85	≤ 0.001**	2.769	1.771 to 4.329
B2	65	32.5	80	57.15		1 Ref.	-

Table (4) showed the non- significant relation between the levels of serum lipid and genotypes of Taq 1B polymorphisms, the serum HDL-C level was lower in the genotype B2B2 than the genotypes B1B1 and B1B2, but the serum TG, VLDL-C levels were higher in the genotype B2B2 when it comparison with genotypes B1B1 and B1B2. The other parameters for instance LDL-C and TC were raised in the genotype B1B1 in comparison with B1B2 and B2B2.

Table 4: Levels of serum lipid in the patients according to the polymorphism of CETP Taq1 B

Parameter	B1B1 (44) Mean ± SD	B1B2 (47) Mean ± SD	B2B2 (9) Mean ± SD	*P*. value
HDL-C (mg/dL)	46.704 ± 9.25	45.829 ± 14.952	42.444 ± 5.615	0.629
TC (mg/dL)	234.772 ± 7.346	220.297 ± 52.489	212 ± 3.732	0.399
LDL-C (mg/dL)	154.613 ± 9.807	149.766 ± 37.387	125.444 ± 2.787	0.110
VLDL-C (mg/dL)	40.454 ± 6.362	40.361 ± 17.862	51.777 ± 7.079	0.170
TG (mg/dL)	201 ± 8.770	189.957 ± 6.679	235.555 ± 11.774	0.273

IV. DISCUSSION

There were a few studies available in Iraq about the prevalence of CETP gene polymorphism in hypertensive patients. Therefore, this study may be the first study included the relationship between CETP Taq 1B polymorphism and lipid profile in hypertensive Iraqi patients. Hypertension is one of the main health problems in Iraqi population over the last decade, and the prevalence of the disease had risen in different ages, it is thought to be a complex polygenic disease caused by various genes, each having a minor effect separately or by interactions (28). This study revealed that there was a highly significant levels of the lipid profile parameters TG, LDL-C, and VLDL-C, TC in hypertensive patients comparison to control group, whereas the levels of serum HDL-C was significantly lower, these results were in agreed with (29). The two main risk factors of cardiovascular diseases and atherosclerosis are hypertension and dyslipidemia. Dyslipidemia leads to lipid increase in the lumen of blood vessels rise resistance of blood flow in the vascular system that leads to hypertension (30). HDL-C acts as antioxidant and anti-inflammatory effects which inhibits the cluster of lipids in the vessels, also the role of HDL-C can facilitate the transfer of cholesterol from peripheral tissues to the liver for degradation and excretion (31).

In the group of patients, there was non-significant differences in the lipid levels according to gender (Table 2). The gender-specific association may reveal differences in CETP role in lipid exchange (32), this indicates that sex did not impact on the lipid profile in hypertensive patients. The absence of significant differences may be due to the insufficient number of participants. There was also no significant difference in HDL-C level among three genotypes (B1B1, B1B2, B2B2) in hypertensive patients, which was probably due to lifestyle and genetic-environmental interactions which may affect the link between lipid levels and the polymorphism of the gene in different populations. In HDL-C variability, the effect of genetic factors was 76 %, where CETP Taq1B polymorphism was assessed to be responsible for 5.8 %. This shows the absence the effect of single nucleotide polymorphism (SNP) on lipid levels because of the role and variations of other SNP of the CETP gene in addition to type of the eat or diet that cause different findings (33).

The frequency of Taq1B polymorphism showed that B2B2 genotype was significantly lower in patients

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group compared with control group ($p< 0.05$). This suggests that the genotype has a protective effect on dyslipidemia. It was confirmed by the odds ratio of the B1B1 which was (2.769) compared to B2B2. These results were in agreement with other studies (34, 35). Lipids and lipoproteins can be regulated by genes in humans, including lipoprotein lipase, apo B, apo E, apo AI, apo AII, apoC, and CETP genes, due to their central role in lipid metabolism regulation (17). CETP behavior affects the HDL-C molecule as well as facilitates the interchange between triglyceride-rich lipoproteins of cholesterol esters of triglycerides and HDL-C. In fact, the HDL-C particle was involved in the transport of reverse cholesterol (36). There was unclear mechanism weather CETP levels were correlated with blood pressure. Results of this study are hypothesizing several possibilities. First, low levels of CETP may have protective effects on blood pressure that was observed because they avoid atherosclerosis by modifying lipoprotein profiles and maintaining dispensability of the arterial wall. Second, HDL cholesterol can affect the endothelial function independently. The CETP was also expressed in endothelial cells, and the effect on endothelial function may be directly modulating (37).

There were some limitations in this study. First, before lipid profile test, no one can interfere with the daily intake of patients that could influence the relationship between CETP gene polymorphism and hypertension. Second, only one SNP of the CETP gene was studied.

This study concluded that there was a quite clear relationship between Taq1B CETP polymorphism and dyslipidemia in Iraqi hypertensive patients, and the B1B1 genotype of the Taq1B CETP polymorphism, as well as B1 allele, were considered as a potential risk factor for hypertension. More future studies with larger sample sizes may performed to support these findings.

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