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Original article

Differences in the interaction between CETP Taq1B polymorphism and dietary fat intake on lipid profile of normolipedemic and dyslipidemic patients with type 2 diabetes mellitus

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SUMMARY

Background & aim: Dyslipidemia is one of the major complications in patients with type 2 diabetes mellitus (T2DM). Dietary fat intake and genetic factors including *CETP* Taq1B polymorphism could also affect lipid profile concentrations, in particular HDL-c. We decided to study the frequency of this polymorphism and its interaction with dietary fat intake on HDL-c concentration among Iranian T2DM patients with and without dyslipidemia.

Methods: In this comparative study, serum samples were collected from 55 patients with dyslipidemia and 129 patients without dyslipidemia. Validated semi-quantitative FFQ was used for food consumption data. *CETP* Taq1B polymorphism was studied by polymerase chain reaction-restriction length polymorphism (PCR-RFLP). We used χ^2 and two-way ANOVA tests for statistical analysis.

Results: The frequency of B1B1 genotype was higher in patients with dyslipidemia ($p = 0.01$). There was no significant relationship between *CETP* Taq1B polymorphism and lipid profile concentrations. In patients without dyslipidemia, the interaction between the polymorphism and total fat intake on HDL-c concentration as well as TG/HDL ratio was significant ($p = 0.02$ and $p = 0.009$ respectively). This was more evident in B1B1 genotype. Moreover, HDL-c concentration was significantly higher in B2B2 genotype with low total fat intake.

Conclusion: Higher total fat intake may affect the relationship between *CETP* Taq1B polymorphism and HDL-c concentration in patients with normolipidemic T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a multifactorial disease [1]. The development of the disease is affected by a combination of lifestyle, environmental and genetic factors [1]. As a global prevalent disorder, around 285 million people, aged between 20 and 70 years, were diabetic in 2012. According to WHO, the incidence of diabetes is rising and the number might extend to 439 million patients in 2030 [2].

Dyslipidemia, as an important risk factor for atherosclerosis, is considered as one of the major complications in T2DM patients [3].

List of abbreviation: T2DM, type 2 diabetes mellitus; CETP, cholesteryl ester transfer protein; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; VLDL, very low density lipoprotein; TG, triglyceride; TC, total cholesterol; CE, cholesterol ester; SNP, single nucleotide polymorphism; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; ANOVA, analysis of variance; ANCOVA, analysis of covariance; BMI, body mass index; WC, waist circumference; CHD, coronary heart disease.

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In these patients, dyslipidemia is characterization by increased concentration of triglyceride (TG), very low density lipoprotein (VLDL) and small dense low density lipoprotein cholesterol (LDL-c) together with decreased concentration of high density lipoprotein cholesterol (HDL-c) [4]. It was reported that even 1% increase in HDL-c concentration is associated with 2–3% reduction in cardiovascular diseases incidence [5]. In general, plasma level of lipoproteins is regulated by environmental factors, such as dietary fat intake [6,7], as well as regulatory genetic factors, including cholesteryl ester transfer protein (CETP) [8]. CETP plays a regulatory role in exchanging the neutral lipids (cholesteryl ester (CE) and TG) between HDL-c and Apo B-containing lipoprotein, such as VLDL and LDL which leads to the increase of atherogenicity and HDL-c catabolism. Moreover, CETP contributes in reverse cholesterol transfer (RCT) by the transfer of CE from HDL3 to HDL2, and facilitates the transfer of CE from peripheral tissues to the liver [9]. Small dense HDL might raise the risk of cardiovascular diseases [10]. Similar to HDL-c concentration, CETP activity varies among normolipidemic people and influenced by genetic [11] and environmental factors including dietary fatty acids intake [12].

Several single nucleotide polymorphisms (SNP) could also affect the CETP activity. One notable example is Taq1B polymorphism in CETP gene (rs708272, nucleotide 277 of intron 1) which is reported to play a role in lipid profile regulation. The frequent allele, B1, has a restriction site for TaqI endonuclease, which is absent in B2 allele [11,13]. In some studies, a reverse relationship was observed between B2 allele and CETP activity [14]. In different studies, the relationship between *CETP* Taq1B polymorphism and dietary fat intake on HDL-c concentration led to controversial results. Li et al. reported a strong relationship between this SNP and total fat, animal fat, saturated fat, and monounsaturated fat [15]. However, Aitken et al. did not observe any significant relationship [16].

In the present study, we aimed to investigate the relationship between *CETP* Taq1B polymorphism and HDL-c concentration via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method in Iranian patients with T2DM and dyslipidemia background. Moreover, we studied whether this relationship is affected by dietary fat intake.

2. Materials and methods

2.1. Study population

This study is part of a larger cross sectional investigation on 816 T2DM patients [17,18], of which we selected 184 patients according to our inclusion criteria: individuals with (TC $\geq$ 200 mg/dl and TG $\geq$ 150 mg/dl) [4] as the dyslipidemic group; and individuals with (TC < 200 mg/dl and TG < 150 mg/dl) for the normolipidemic group. Patients taking lipid-lowering drugs were excluded. In total, 55 patients with dyslipidemia and 129 patients with normal lipid profile were enrolled. The collection of demographic information, dietary and METs information, anthropometrics measurements, and biochemical tests were performed according to Noorshahi et al. [17].

We obtained written informed consent from our participants and the study including ethical permit (IR.TUMS.REC.1395.2815) approved by Tehran University of Medical Sciences.

2.2. Genotyping

DNA was extracted from whole blood by salting-out method, as previously described [19]. Taq1B polymorphism genotyping was determined using PCR-RFLP method. PCR was carried out in 25 μl total volume with 2X Master mix (Ampliqon, Copenhagen, Denmark), 0.6 μM of each primer (Forward: 5′-CACTAGCCCAGAGAGAGGAGTG-3′; Reverse: 5′-TGAGCCCAGCCG-CACACTAAC-3′), and 75 ng genomic DNA. Amplification was performed in 35 cycles: denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 40 s. PCR products were digested by Taq1 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 65 °C for 30 min. Restriction products were analyzed by 2% agarose gel electrophoresis.

2.3. Statistical analysis

Data was analyzed using SPSS (version 16; SPSS Inc., Chicago, IL). The normality of data was tested by Kolmogorov–Smirnov and log transformation. Chi-square (χ^2) test and logistic regression were utilized for comparing the genotype and allele frequency. One-way ANOVA was used for lipid profile comparison among three genotypes (B1B1, B1B2, and B2B2) and in each group, separately. In addition, two-way ANOVA were used for interactions and ANCOVA for energy, total fat intake, and waist circumference (WC). P-value <0.05 was considered as statistically significant.

3. Results

Statistical analysis of the characteristics of patients revealed that the difference between the baseline characteristics and anthropometric measurements of dyslipidemic and normolipidemic subjects were not significant (Table 1). The concentration of LDL-c, TC/HDL, TG/HDL and LDL/HDL were significantly different between the two groups (Table 1).

The frequency of B1B1 genotype in dyslipidemic group was significantly higher than the normolipidemic group (P = 0.01) (Fig. 1). In both groups, there were no relationship between *CETP* Taq1B polymorphism and lipid profile concentrations (Fig. 2). However, in both groups, B2B2 patients had higher HDL-c concentration than two other genotypes (Fig. 2).

We considered the percentage of energy from each fat type. In normolipidemic group, the interaction between *CETP* Taq1B polymorphism and total fat intake on HDL-c concentration and TG/HDL was significant (P = 0.02 and P = 0.009, respectively) (Table 2) and the interaction was more evident in B1B1 genotype (Fig. 3). However, no interaction was found in any genotype of the dyslipidemic group (Table 3).

For both groups, we divided the fat consumption into two categories of high and low fat intake. In normolipidemic group, there was a significant relationship between genotype and TG/HDL ratio among patients with high fat intake (P = 0.02). Moreover, the HDL-c concentration was significantly higher in low fat intake patients with B2B2 genotype, in comparison with B1B1 genotype (P = 0.03) (Table 2, Fig. 3). Although in dyslipidemic group, the interaction between genotype and monounsaturated fatty acid (MUFA) intake on TG/HDL ratio was significant, however it did not remain significant after adjusting for energy. No other significant relationship was found between genotype and lipid profile with the intake of different amounts of saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA).

4. Discussion

The aim of the current study was to investigate the interaction between *CETP* Taq1B polymorphism and dietary fat intake on HDL-c concentration in normolipidemic and dyslipidemic patients with T2DM. In dyslipidemic group, the frequency of B1B1 genotype was significantly higher than the normolipidemic group. We found that B1B1 genotype might be a risk factor for dyslipidemia in diabetic patients. Consistent to our findings, Wu et al. study on individuals with coronary heart disease (CHD) revealed more frequent B1B1

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