Journal of Education for Pure Science- University of Thi-Qar
Vol.13, No.4 (Dec, 2023)
Website: jceps.utq.edu.iq Email: jceps@eps.utq.edu.iq

DOI: http://doi.org/10.32792/utq.jceps.10.01.01

Adiponectin gene polymorphism (rs2241766) in patients with coronary artery disease

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Received 20/11/2023, Accepted 27/11/2023, Published 1/12/2023

Abstract

The present study aimed to determine the association between the adiponectin gene (ADIPOQ gene) and coronary artery disease (CAD). Sixty individuals (40 patients and 20 healthy) were comprising in this study. The current results demonstrated significant relationship between TG genotype (22.5% ofCAD patients and 30% of healthy individuals) when compared with TT genotype. GG genotype was less common (12.5% and 15% of patients and control), as comparing with other genotypes. Also, there is no significant differences between GG genotype and TT genotype. There is no significant differences in allel frequencies between CAD patient and control group, while, T allel showed high frequency rate (76.25% and 70% in patients and control group respectively). Five mutations were determined within the studied region of the ADIPOQ gene using mutation surveyor program: 1 (20%) Deletion (g. 15324del C), 2 (40%) Transversion (g.15430G>T), 1 (20%) Transversion (g.15345A>C), 1 (20%) Transversion (g.15454T>A). In conclusion,the current study showed that rs2241766 polymorphisms affect in patients with coronary artery disease.

Keywords: ADIPOQ gene, polymorphism, coronary artery disease. .

Introduction

Cardiovascular disease(CVD) is one of the leading issues to death in the world. One study appraised that the rate of mortality caused by CVD in the world rise from 17.1 million in 2004 to 23.4 million in 2030. Coronary artery disease (CAD) is one of the most common cardiovascular diseases [1]. CAD involves genetic and environmental factors and their interactions. Traditional risk factors account for more than half of the prevalence of CAD, and despite attempts conducted to establish the molecular and genetic determinants that may be accountable for variations in CAD, the etiology and complex multigenic bases of atherosclerosis are still not completely understood [2,3].

To date, the precise pathogenesis of CAD remains mainly unknown. Nevertheless, abundance of evidences exhibited that genetic factors are very

important for the development of CAD. First, family clustering of CAD was indicated widely, and past twin studies demonstrated that the genetic mark of CAD was over 50 % [4]. Second, multiple genetic variants were established to be linked with an increased susceptibility to CAD by prior genetic association studies ,and screening of frequent causal variants was also demonstrated to be an effective method to predict the individual risk of developing CAD [5].

Adiponectin (ADIPOQ), an adipocytokine that manages energy and material metabolism, is involved in the development of various metabolic disturbances comprising type II diabetes and obesity, and it was evident that these two common metabolic disorders were linked with raised risk of CAD [6]. Adiponectin is one of the most intensely debated secretion products of white fat cells that has been involved increasingly in the pathogenesis of atherosclerosis and in insulin resistance, and data on the prospective impacts of adiponectin plasma-concentration determination in cardiovascular diseases in humans are evolving. Some clinical studies have showed strong associations between low plasma adiponectin levels and CAD [7]. In addition, the expression level of adiponectin was too significantly reduced in CAD patients [8]. Therefore, functional ADIPOQ genetic polymorphisms, which may alter the expression level of adiponectin, may also affect individual susceptibility to CAD. some pilot studies already investigated associations of two common functional ADIPOQ polymorphisms, rs1501299 and rs2241766, with the susceptibility to CAD [9].

Materials and Methods

This study was conducted in Al-Nasiriyah Heart Center and biotechnology unit in Mazaya college for the period from November 2022 to the end of April 2023. Sixty individuals (40 patients and 20 healthy) were included in the present research. patients group were selected randomly from Al-Nasiriyah cardiology disease.

Sample collection

Approximately 2.5 ml of venous blood samples were collected from participants. The blood specimens were placed in the EDTA vacutainer tubes for genomic DNA extraction, The genomic DNA was stored at -20 c freezing , then used to amplification of ADIPOQ gene.

DNA isolation and Genotyping.

Genomic DNA was isolated using SYNCTMDNA Mini kit from white blood cells. ADIPOQ polymorphism was determined using sequencing method. Forward primer: 5-CTGCTGAGATGGACGGAGTC-3 and reverse primers 5- GTCTGTGATGAAAGAGGCCAGA -3to amplify a 348bp fragment as shown in Fig.1. The PCR program was initial denaturation at 95˚C for 10 min followed by 30 cycles of 95˚C for 30 sec., 58˚C for 30 sec. (annealing) and 72˚C for 35 sec. (extension). The reaction was completed by a final extension cycle at 72˚C for 10 min. Amplified product was sent to a Macrogen Company to analyze the nucleotide sequence using a Genetic analyzer device.

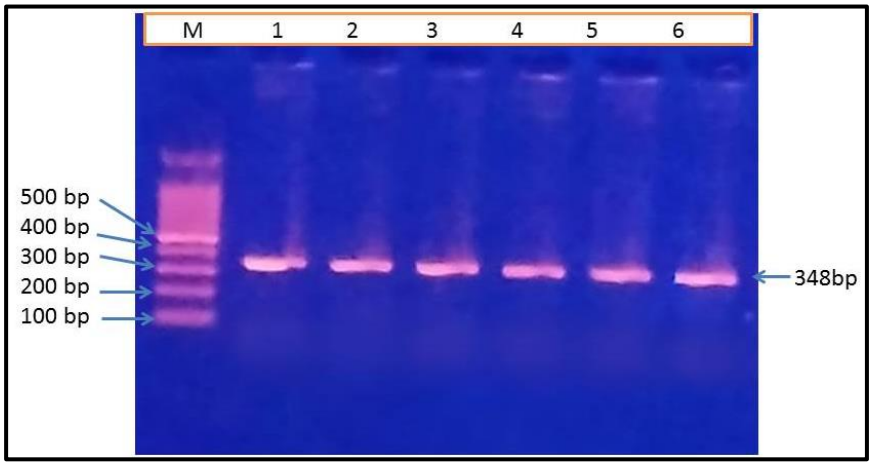

Figure (1) PCR products of ADIPOQ gene which analyzed on 2% agarose gel.

Statistical Analysis

All statistical analysis was conducted using version 20 of SPSS. Odd ratio was used to compare the frequencies of genotype between patients and control group. OR≥ 0.05 were considered statistically significant.

Results

To determine the nucleotides sequenceand identification of mutations in the studied region of Adiponectin gene, the PCR product was sent to the Macrogen company\ Korean. The allele frequencieswere determined as shown in Figure (2).

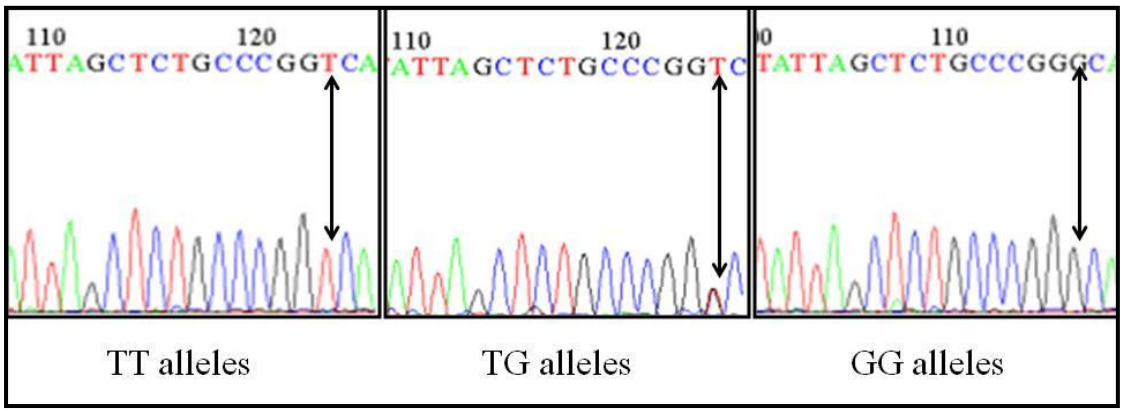

Figure (2) Allele frequencies in ADIPOQ gene polymorphism (rs2241766).

The current study showed that the TT genotype was common in patients and control group. 22.5% of patients and 30% of control group have TG genotype with significant differences compared to TT genotype (OR=1.57 , CI 95% 0.45 – 5.50). However, 12.5% and 15% of patients and control group, respectively have GG genotype with no significant differences compared to TT genotype(OR=1.42 , CI 95% 0.28 – 6.99). The present study found that the frequency of T allel was 76.25% and 70% in patients and control group respectively. There is no significant differences in allel frequencies between patents and control group.

Table (1) Percentage of TT, TG, GG genotype in CAD patients and control and allel frequency.

Genotype	Patients N (%)	Control N (%)	OR	95 % CI
TT	26 (65%)	11 (55%)	1.00	———
TG	9 (22.5%)	6 (30%)	1.57*	0.45 – 5.50
GG	5 (12.5%)	3 (15%)	1.42	0.28 – 6.99
Total	40 (100%)	20 (100%)	———	———
T allele	61 (76.25%)	28 (70 %)	1.00	———
G allele	19 (23.75%)	12 (30 %)	1.37	0.58 – 3.21
Total	80 (100%)	40(100%)	———	———

95% CI, Confidence Interval. OR, Odds ratio.

The nucleotide sequences of the amplification fragment were alignment with reference sequence of the ADIPOQ gene (NG_021140.1) using mutation surveyor program shown in Figure 3. By alignment, it was found that there were five mutations in the studied region of the ADIPOQ gene, as shown in Table (2).

Table (2) Mutation in ADIPOQ gene in CAD patients.

Mutation	Type	Frequency	Effect
g. 15324del C	Deletion	1 (20%)	Frameshift
g.15430G>T	Transversion	2 (40%)	Silent mutation
g.15345A>C	Transversion	1 (20%)	--
g.15454T>A	Transversion	1 (20%)	His>Gln

Figure (3) Alignment with reference sequence using mutation surveyor program.

Discussion

Adiponectin was found to play a momentous role in the diabetes mellitus, metabolic syndrome, and coronary artery disease among different populations according to other studies [10,11]. The present study demonstrated that high rate of genotype TT (65%) in patients with CAD then genotypes TG (22.5%) and GG(12.5%), Also, the rate of the T allele is (76.25%) more than the allele G (23.75%). The present results agree with study of Diah et al. [12] who showed that the rate of genotypes TT, TG and GG were (63.3%), (26.6%) and (10%) respectively, as well, the rate of the T allel is (76.6%) and G allel (23.3%) and this is comparable to the present study. The results of this study suggested that there is significant association between the genotype TG of the ADIPOQ gene in patients with coronary artery disease, and this results were confirmed by Al-Daghri *et al*. [13] who found that there is a significant relationship between SNPs 45T>G polymorphism in diabetic Saudi population with CAD, while, Eissa [14] proposed that there is no important association between the +45T>G of the ADIPOQ and cardiovascular conditions in Saudi population. The genetic variants may be affected in the expression level of the ADIPOQ gene in CAD patients. Piestrzeniewicz et al. [15] and Persson *et al.* [8] reported that the expression level of adiponectin was also significantly decreased in CAD patients. Dai et al. [5] showed that the different genetic variants were to be linked with an increased susceptibility to CAD by prior genetic correlation studies, and screening of common causal variants was also indicated to be an efficient way to predict the individual risk of developing CAD.

In this study, genetic variations represented by five mutations were observed in different positions of adiponectin gene may be associated with CAD patients. Many variations in the adiponectin gene were associated with various diseases, like Type 2 Diabetes Mellitus T2DM, [16] metabolic syndrome [17] and CAD [11]. The present results were confirmed by Lacquemant *et al*. [18] who mentioned that polymorphism rs2241766 was linked with an increased risk of coronary artery disease among with type 2

diabetic patients, Bacci *et al*. [19] showed that polymorphism rs1501299 was linked with a decreased risk of coronary artery disease. Zhang *et al*. [20] reported that the associations between rs2241766, rs1501299 and rs266729 in the ADIPOQ and CVD were significant but weak, as well, High quality studies are still needed to approve the associations, particularly for rs2241766.

Conclusion:

The current study showed that rs2241766 polymorphisms regarded as considerable rsck factor in patients with coronary artery disease.

Ethical clearance

The research and laboratory tests were approved by the Ethics Committee from the Al-Nassiriyah cardiology center .

Source of funding : self

Conflict of Interest : null

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