ORIGINAL ARTICLE

Analysis of two single-nucleotide polymorphisms (rs2241766 and rs1501299) of the adiponectin gene in patients with coronary artery disease and coronary slow flow

Muhammad Diah[1,2], Aznan Lelo[3], Zulfikri Muhktar[4], Dharma Lindarto[5]

[1]Division of Cardiology, Department of Internal Medicine, School of Medicine, Syiah Kuala University/Dr. Zainoel Abidin General Centre Hospital, [2]School of Medicine, Universitas Sumatera Utara, [3]Department of Pharmacology, [4]Department of Cardiology, [5]Division of Endocrine Metabolic; Department of Internal Medicine, Universitas Sumatera Utara

ABSTRACT

Aim To investigate the association of two single-nucleotide polymorphisms (SNPs) of the adiponectin gene (+45 T>G [rs2241766] and +276 G>T [rs1501299]) in patients with coronary artery disease (CAD), coronary slow flow (CSF) and in healthy subjects.

Methods The study investigated 90 patients: 30 patients with coronary artery disease (the CAD group), 30 with only coronary slow flow (the CSF group) and 30 healthy patients. Genotyping was carried out for two of these SNPs in the adiponectin gene using the TaqMan polymerase chain reaction (PCR) method.

Results There were no significant differences in the frequencies of polymorphism +45T>G (rs2241766) genotype (p=0.464) and the allele (p=0.362). There were also no significant differences between the +276 G>T (rs1501299) genotype and the allele (p=0.624 and p=0.281, respectively).

Conclusion Our study indicated that in patients with CAD, CSF, and in healthy subjects, the SNP +276G>T and +45T>G alleles of the adiponectin gene were not associated.

Key words: genetic association, cardiovascular disease, coronary angiography

Corresponding author:
Muhammad Diah
School of Medicine,
Universitas Sumatera Utara
Dr. Mansyur Street No. 5, Padang Bulan,
Medan, Indonesia
Phone: +62 811 682 2238;
E-mail: Muhammaddiah6262@yahoo.com
ORCHID ID: https://orcid.org/0000-0002-1183-8621

Original submission:
27 November 2019;
Revised submission:
28 January 2019;
Accepted:
31 January 2019
doi: 10.17392/1102-20

Med Glas (Zenica) 2020; 17(2): 341-345

INTRODUCTION

Coronary artery disease (CAD) is one of the most common cardiovascular diseases. The World Health Organization has issued several reports indicating that since 2008, cardiovascular disease (CVD) has become the principle cause of death worldwide. One report estimated that mortality caused by CVD would rise from 17.1 million in 2004 to 23.4 million in 2030 (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). Adiponectin is one of the most intensively discussed secretion products of white fat cells that has been implicated 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. Several clinical studies have demonstrated strong correlations between low plasma adiponectin levels and CAD (4).

It is therefore possible that functional genetic polymorphisms that may alter the expression level of adiponectin may also affect individual susceptibility to CAD. Two of the most commonly studied SNPs are the silent T to G substitution in exon 2 (+45T>G) and the G to T substitution in intron 2 (+276G>T). However, inconsistent findings regarding the association of these two SNPs, either independently or as a haplotype have been reported (5, 6). The two SNPs +45T>G19–22 and +276G>T 23–25 have been repeatedly found to correlate with CAD, albeit with some controversial studies concluding that there was no association with CAD and related complications, findings that may be due to differences in ethnic populations studied (7). To clarify these conflicting results, the data regarding the association between adiponectin polymorphisms and CAD require further investigation.

Aim of this study was to investigate the association between two single-nucleotide polymorphisms (SNPs), +45 T>G (rs2241766) and +276 G>T (rs1501299) of the adiponectin gene in patients with CAD, CSF, and in healthy subjects.

PATIENTS AND METHODS

Patients and study design

The study was conducted between December 2017 and February 2018 in the Division of Cardiology, Department of Internal Medicine, School of Medicine, University Syiah Kuala and the Cardiac Catheterization Laboratory at Dr. Zainoel Abidin Regional General Hospital, Banda Aceh, Indonesia.

Patients involved were aged 26 years and above and were consecutively recruited. Patients who had experienced clinical symptoms of chest pain with typical angina problems were divided into CAD and CSF groups, based on the results of coronary angiography. Patients who did not have chest discomfort as confirmed by electrocardiography and had not had coronary angiography were healthy group. Patients with total stenosis of coronary arteries and coronary artery bypass grafts were excluded. Patients with significant comorbidities, including hypertension, diabetes mellitus, the use of anti-inflammatory drugs other than aspirin, renal or hepatic dysfunction, body mass index (BMI) of >22.9 kg/m^2 or <18.5 kg/m^2, smoking, and alcohol consumption, were excluded from the healthy group.

The protocol of this cross-sectional study was approved by the Ethical Review Committee, School of Medicine, Universitas Sumatera Utara, Medan, Indonesia. Prior to the study, all patients provided written informed consents.

Methods

Venous blood samples were taken and tested for hemoglobin, hematocrit, white blood cells, platelets, erythrocyte sedimentation rate, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, urea, creatinine, uric acid, fasting glucose, and 2-hour post prandial glucose, using standard methods.

Coronary angiography and thrombolysis in myocardial infarction (TIMI). The standard Judkins technique was employed to perform coronary angiography of the participants (8). The angiography was recorded at the left anterior oblique, cranial, right anterior oblique, caudal, and horizontal positions. These examinations were carried out by two cardiologists who had

not had any information about the clinical characteristics of the patients. In addition, the coronary artery flow of the patients was assessed using the TIMI frame-count method (9) and the differences between the first and the last frames in the count were calculated. The cut-off values for the length for normal visualization of coronary arteries were 36.2±2.6 frames for the left anterior descending artery (LAD), 22.2±4.1 frames for the left circumflex artery (LCx), and 20.4±3 frames for the right coronary artery (RCA). The corrected cut-off value for the LAD was 21.1±1.5 frames. The mean TIMI frame count for each subject was calculated by dividing the sum of the TIMI frame counts for LAD, LCx, and RCA by three.

Determination of adiponectin genotyping. Genomic DNA was extracted from 5 mL of whole blood using a commercially available DNA extraction kit **(Genomic DNA Mini Kit, Geneaid, USA)** according to the manufacturer's instructions. Researchers screened two SNPs in rs2241766 and rs1501299 of the adiponectin gene in all patients with assays using quantitative real-time TaqMan PCR (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Conformity to normal distribution of the continuous variables was examined using the Shapiro–Wilk test. Parametric or non-parametric tests were performed based on the data distribution and the number of groups, and *post hoc* comparisons were conducted using suitable testing. Paired-sample *t*-testing was used to compare dependent groups. Genotype and allele frequencies for +45 T>G (rs2241766) and +276 G>T (rs1501299) polymorphisms of the adiponectin gene were determined by direct counting. Statistical comparisons between the three groups were performed by applying the chi-squared test and Fisher's exact test with $p<0.05$ being considered as statistically significant.

RESULTS

The study comprised 90 patients: 30 in the CAD, 30 in the CSF and 30 in the healthy group (Table 1).

Based on collected clinical data, there was a statistically significant difference between the CAD, CSF, and the healthy groups in terms of age, sex, and systolic blood pressure (SBP) ($p<0.001$). Total cholesterol, serum triglycerides, and creatinine were significantly higher in patients with CAD compared to the patients with CSF and the healthy ones ($p<0.001$).

There was no significant difference in the TIMI frame counts for RCA, LCx, LAD, corrected TIMI frame count (cTFC) LAD, and cTFC between the groups. The most common target vessel in the CSF patients was RCA (93.3%) (Table 2).

The distribution of +45 T>G and +276 G>T alleles and genotypes was comparable between the patients with CAD, CSF, and the healthy group. In 90 patients, there were no significant differences in the frequencies of polymorphisms of +45T>G

Table 1. Demographic and clinical characteristics of coronary artery disease (CAD), coronary slow flow (CSF) and healthy patients

Variable	CAD (n=30)	CSF (n=30)	Healthy (n=30)	p
Age (years) (No, %)	55 (37-65)	52 (37-65)	35 (27-51)	<0.001
Male female (No, %)	29 (96%) /1 (4%)	10 (33%) / 20 (67%)	17 (56%)/13 (44%)	<0.001
BMI (kg/m2) (mean±SD, median minimum-maximum)	23.7 (21.6-29.0)	22.7 (18.1-31.2)	23.2 (18.5-25.1)	0.08
SBP (mmHg)	130 (112-152)	126.50 (109 -147)	116.50 (107-128)	<0.001
DBP (mmHg)	84.30±7.13	84.93±9.63	78.10±8.84	0.004
Hemoglobin (g/dL)	14.2 (10.1-17.1)	13.2 (8.7–16.6)	13.2 (11.6-17.5)	0.009
Hematocrit (%)	42.17±4.93	39.90±4.85	39.93±3.44	0.085
WBC (uL)	8350 (5900-14900)	8700 (4600-16600)	7700 (4500-10100)	0.039
Platelet (103/uL)	263.4±59.4	270.7±74.2	278.1±65.2	0.697
Neutrophil-lymphocyte count ratio	1.6 (0.8-19)	1.3 (0.8-5.1)	1.3 (0.8 -1.7)	0.002
Total cholesterol (mg/dL)	198 (112-461)	194 (127-336)	149 (90-198)	<0.001
LDL cholesterol (mg/dL)	50.4±18.31	49±17.7	64.8±14.5	0.001
HDL cholesterol (mg/dL)	121.5 (30-225)	129 (50-252)	111 (62-156)	0.623
Triglyceride (mg/dL)	143.5 (88-231)	106 (55-373)	94 (21 -129)	<0.001
Urea (mg/dL)	30.9±11.03	23,8±6,62	23.3±5.20	0.004
Creatinine (mg/dL)	1.0 (0.6-1.6)	0.8 (0.6 -1.3)	0.7 (0,5 – 1,0)	<0.001
Fasting glucose (mg/dL)	101 (71-218)	109 (89 – 154)	98 (82 – 117)	0.013
2 hours post prandial glucose (mg/dL)	129 (100 – 226)	133 (103 -226)	126 (97 -176)	0.214

Data were presented as mean±SD, median (minimum-maximum) or n (%); BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ESR, erythrocyte sedimentation rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein

Table 2. Thrombolysis in myocardial infarction (TIMI) frame counts of coronary artery disease (CAD) and coronary slow flow (CSF) patients

Variable	CAD (n=30)	CSF (n=30)	p
TIMI frame (mean±SD)			
RCA	28.07±8.82	26.83±3.87	0.486
LCX	34.4±10.34	28.37±2.76	0.003
LAD	47.27±10.11	42.37±7.77	0.04
cTFCLAD	27.8±5.9	24.67±4.8	0.029
cTFC	30.13±7.94	26.83±3.553	0.042
Target vessel (No, %)			
RCA	22 (73.3)	28 (93.3)	0.038
LCx	4 (66.6)	25 (83.3)	0.136
LAD	26 (86.6)	25 (83.3)	0.718

RCA, right coronary artery; LCx, left circumflex artery; LAD, left anterior descending artery; cTFC, corrected thrombolysis in myocardial infarction frame count

(rs2241766) genotype (p=0.464) and allele (p=0.362) in all three groups of patients (Table 3).

Table 3. Adiponectin +45T>G (rs2241766) genotype and allele distribution in patients with coronary artery disease (CAD), coronary slow flow (CSF) and healthy patients

SNP	Genotype	No (%) of patients			p
		CAD (n=30)	CSF (n=30)	Healthy (n=30)	
+45T>G	TT	19 (63.3)	21 (70)	22 (73)	0.464
	TG	8 (26.6)	6 (20)	8 (26)	
	GG	3 (10)	3 (10)	0 (0)	
Allele	T	46 (76.6)	48 (80)	52 (86.6)	0.362
	G	14 (23.3)	12 (20)	8 (13.3)	

SNP, single-nucleotide polymorphism

In terms of the frequency of +276 G>T (rs1501299) genotype and allele, there was no significant difference (p=0.624 and p=0.281, respectively) (Table 4).

Table 4. Adiponectin +276 G>T (rs1501299) genotype and allele distribution in patients with coronary artery disease (CAD), coronary slow flow (CSF) and healthy patients

SNP	Genotype	No (%) of patients			p
		CAD (n=30)	CSF (n=30)	Healthy (n=30)	
+276G>T	GG	16 (53.3)	17 (56.6)	12 (40)	0.624
	GT	10 (33.3)	11 (36.6)	13 (43.3)	
	TT	4 (13.3)	2 (6.7)	5 (16.7)	
Allele	G	42(70%)	45 (45)	37 (61.6%)	0.281
	T	18 (30)	15 (25)	23 (38.3)	

SNP, single-nucleotide polymorphism

DISCUSSION

CSF has demonstrated to be more common in males, smokers, and individuals with hyperlipidemia, metabolic syndrome, and/or obesity. There have been many studies conducted by examining clinical features of CSF, such as the one conducted by Yilmaz et al. in a Turkish population (10). In this study, BMI, glucose levels, lipid derangements, and metabolic equivalents (METs) were identified as significantly associated with CSF. In our study, age, sex, BMI and SBP were significantly different. There were also statistically significantly differences in the parameters for lipids and creatinine.

Results of the present study showed that the most common target vessel in the CSF group was RCA. These results seem to be in accordance with previous reports on angiographic characteristics in patients with CSF, and RCA has been identified as the predominant vessel involved in CSF (11). However, another study found that the most common artery involved was LAD, followed by LCX, and RCA (12).

Adiponectin is a 244-amino-acid protein secreted exclusively by adipocytes. This protein has been proposed as protecting against CVD via its metabolic anti-inflammatory effects mediated by crosstalk between the cAMP-PKA and the NF Kappa B signaling pathways (13). As found in the study by Matsubara et al, adiponectin also stimulated fatty-acid oxidation, decreased plasma triglycerides (TG), and improved glucose metabolism by increasing insulin sensitivity. However, the lack of consistent data on the association between adiponectin and its genetic variants and the risk of CAD means that the precise role of this protein in CAD and CVD in general is still unclear (14).

Several studies have been conducted into the association of adiponectin polymorphisms with CAD, such as the study by Ohashi et al. who found no association of SNP276 and SNP94 in 383 Japanese patients with confirmed coronary heart disease and 368 healthy controls (15). Lacquemant et al. reported that among 162 Caucasians with type 2 diabetes, SNP+45 showed significant association with increased CAD risk, whereas SNP+276 failed to show any association (16). On the other hand, in a study recently reported by Filippi et al. among 325 CAD patients and 270 members of the control group, SNP+276 showed significant association with CAD (17).

Bacci et al. reported that among 376 Caucasians with type 2 diabetes, SNP+276 showed significant association with CAD. This association was independent of serum adiponectin level (18). A recent study by Jung et al. did not find any correlation between SNP+45 and SNP+276 and the presence of CAD (19).

In our study, there were no significant associations between SNP+45 and SNP+276 in patients with CAD, CSF, or the healthy ones. The difference between our study and the other one is a relatively small number of patients. However, there are no other studies recently conducted or published on polymorphism and adiponectin in patients with CAD and CSF in Indonesia.

In summary, this study found no significant associations between adiponectin polymorphisms +45 T>G and +276 G>T in patients with CAD, CSF, and healthy subjects. The researchers suggest that further studies be conducted with larger study populations.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Competing interests: None to declare.

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