Association of Adiponectin Gene Polymorphism with Coronary Artery Disease in Obese Patients

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Abstract

Background The relation of Two single nucleotide polymorphisms (SNPs) at the adiponectin locus(G/T) with coronary artery disease (CAD) is controversial. Some studies confirmed associations and some did not.

objectives of the present case control study were to determine whether any association between the coronary artery disease (CAD) in obese patients and Adiponectin Gene Polymorphism. Comparison between obese coronary artery patients.

Methodology of extracted DNA obtained from the studied samples was genotyped using Polymerase Chain Reaction (PCR) and sequincing. The genotype assay was used for the analysis of the adiponectin gene (ADIPOQ) G276T rs1501299 for testing the hypothesis by analyzing the genotypes for single nucleotide polymorphism (G276T) in the ADIPOQ gene.

Result Adiponectin at rs1501299 and their allele frequencies were similar between the study and the control groups ($p>0.05$) while the same study found that adiponectin rs2241766 gene polymorphism and allele frequency were statistically significantly lower in the study group ($p<0.05$). this contrast may be due to different of genes studied by the current work which was G276T.

Conclution The findings of this study revealed that Difference between the groups (control and coronary artery cases) concerning the type of Pholymorphysism (Homozygote & Heterozygote) was insignificant.

Keywords Obesity, Adiponectin, Coronary artery disease (CAD), adiponectin gene (ADIPOQ) G276T

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Introduction

Adiponectin is viewed as an insulin-sensitizing hormone with anti-inflammatory [1]and anti atherogenic effect [5]. In accordance, plasma adiponectin is decreased in metabolic disorders including type II diabetes mellitus (T2DM). However, in spite of the apparently beneficially effects, recent data from large prospective studies have consistently linked high adiponectin levels with increased cardiovascular (CV) disease and mortality[6]. Adiponectin has beneficial effects in the cardiovascular system by directly acting on the component cells of the heart and blood vessels .[7] the associations between adiponectin gene in the ADIPOQ and CVD were significant according to [8].

Obesity is a condition in which the number and size of adipocytes increases with further increase of the total fat mass. With industrialization, obesity is advancing along with its association with the risk of diseases such as dyslipidemia, insulin resistance, high blood pressure (HBP), and eventually atherosclerosis or other cardiovascular diseases. Although the risk is well established, the mechanisms leading to it are still unclea[9] . Obesity has increased the rate of diet –releated disease such as hypertention , diabetes type2 and cardiovascular disease [10]. The pathogenic relationship between obesity, metabolic syndrome, and their cardiovascular complications is well established; however, the mechanisms by which excess body fat causes these conditions need to be

clarified. The direct vascular and metabolic effects of plasma proteins that from originate adipose tissue, especially adiponectin, have received growing attention [11].

Coronary artery disease in the spectrum of cardio vascular disease plays predominant role, acconting for one third to half of all cases ,constituting aserious public health problem thatis one of the leading causes of mortality worldwide [12]. Adiponectin is the product of of AMP1(adiponectin) gene wich is approximately spans 15.8 kb [13]. And compossed of 244 amino acids and 30 KDa protein with C-terminal globular domain and collagen-like N-terminal domain that allows for hexameric formation befor being secreation.[14]. AdipoQ has been linked to susceptility locus for metabolic syndrome, type 2 diabetes millites and cardiovascular disease[15]. Single nucleotide polymorphism (SNPs) of adipoQ is mapped on chromosome 3q27 and is composed of three exons [16] polymorphism +276G>T (rs1501299) in second entron have been associated with CAD susceptibility[17].

Materials and Methods

This is a case control study. The study was conducted on patients attending Center of Sudanese Heart Center in Khartoum State, The study was approved ethically by research board of faculty of Medical Laboratory Sciences - Omdurman Islamic University, (Appendix 1) and a written informed consent was obtained from all participants..A structured questionnaire was designed to obtain demographic data (Gender, Age, BMI and Duration for CAD patients).

The study sample was performed on obese and overweight patients suspected with Coronary Artery Disease (CAD). Of the 88 patients, 62 were male (aged 22to 61 years) and 26 were female (aged 22 to 49 years). The study cases were divided into two groups: Control group (44 cases), Test group (44 cases). The diagnosis of control group was based on obesity without coronary artery disease, whereas the diagnosis of test group was based on obesity with coronary artery diseases.

2.1.1. Patients with obesity and/or coronary artery disease were included in this study (both sex) with Body Mass Index (BMI) above 25 Kg/m who underwent

elective coronary angiography for the investigation of the existence of chronic stable CAD. Patient with 50% or greater diameter stenosis in at least one major coronary artery was considered as CAD positive patients, and was classified into two groups. The first group was obese patients suffering from CAD and the second group was obese patients free from CAD. Patients without coronary artery and obesity were excluded from the study. And patient with un stable angina or acute myocardial infarction, un stable condition included infection , heart failure, malignancies, menopause female, renal disease (creatinin level>1.5mg/dl) were excluded.

2.1.2. DNA extraction protocol

DNA was extracted using G-spin TM total DNA extraction kit which as follow: 200µl of whole blood was added into a1.5ml microcentrifuge tube, 20µl of proteinase K and 5µl of RNase A solution into sample tube and was gently mixed. 200µl of buffer BL was added into upper sample tube and was mixed thoroughly, then the lysate was incubated at 56 C for 10 minutes. The 1.5 ml tube was briefly centrifuged to remove drops from the inside of the rid. 200µl of absolute ethanol was added into the lysate and was mixed well by gently inverting 6 time, After mixing the 1.5 ml tube it was briefly centrifuged to remove drops from inside of the lid. The mixture was applied carefully to the spin column (in a 2 ml collection tube) without wetting the rim, cap was closed and centrifuged at 13,000 rpm for 1 min. The filtrate was discarded and the spin column was placed in anew 2 ml collection tube. 700 µl of buffer WA was added to the spin column without wetting the rim and was centrifuged for 1 min at 13.000 rpm. The flow-through was discarded and the collection tube was reused. Buffer WA was added in 700 µl to the spin column without wetting the rim and centrifuged for 1min at 13.000 rpm. The flow-through was discarded and placed the column into a new 2.0 ml collection tube, then centrifuged again for additionally 1 min to dry membrane. Then discarded the flow-through and collection tube altogether. The spin column was placed into a new 1.5 ml tube (not supplied), and 30-100 µl of buffer CA directly into the membrane and incubated for 1 min at room temperature and was centrifuged for 1 min at 13.000 rpm to elute.

DNA samples were genotyped using polymerase chain reaction (PCR) and sequencing.

The genotype assay was used for the analysis of the *ADIPOQ* gene, G276T. Two sets of primers (0.5nmol) from TIB (TIB Molbiol Inc., Germany). For intron 2 region amplification, the forward primer (5′-CTACACTGATATAAACTATATGGAG-3′) and the reverse primer

(5′-CCCCAAATCACTTCAGGTTG-3′) will be used. The reactions will be carried out in a final volume of 25 µl, containing 3 µl genomic DNA (0.2 µg), 5µl Star Taq Master Mix, 20 distilled water and 2.0µl of each primer (0.1 µM). Following the first denaturation for 5 min at 96°C, PCR will carried out for 40 cycles with denaturation at 96°C for 35 sec. The annealing temperature will be at 63°C for G276T SNP and 53°C, this will be carried out for 35 sec and extension at 72°C for 45 sec, with a final extension for 4 min. PCR products that contain G276T SNPs will be digested at 37°C, and electrophoresed on a 1.7% agarosegel (ROWYDA *et al*., 2012).[4]

Gel preparation

PCR products were electrophoresed in a 1.7% agarose gel and visualized using a gel documentation system (SYNGENE, BIO EMAGING). The gel was stained by Ethidium bromid dye. These were run adjacent to 5µl of 100bp DNA ladder RTU (GeneDire, Cat. No.DM012-R500S).

Statistical analysis

Descriptive statistics, and correlation between adiponectin gene polymorphism and cardiovascular obese patients. as well as Odds Ratios (OR) and P. Values were calculated using SPSS (Statistical Package for Social sciences), version 16 for windows.

Results

Descriptive statistics: The frequency and percentage for demographic measures were presented in table1. As shown from the table that 37 (67.7%) of selected CAD patiens were male and 7 (12.3%) were female, 2 (4.5%) cases were 22 – 35 year aged and 42 (45.5%) were > 35 year, 20 (45.5%) cases had body mass index (BMI) ranged between 22 – 35 and 24 (54.5%) cases had BMI > 35, and 5 (11.4) cases showed one year disease history corresponding to 39 (88.6%) cases showed disease history of more than one

year. On the other hand, for obese free CAD (control), 25 (56.8%) cases were male and 19 (43.2) were female, 22 (50.0%) aged 22 – 35 year and 22 (50.0%) aged > 35 year, 25 (56.8%) had body mass index (BMI) of 26 – 35 and 19 (43.2%) had BMI > 35.

Table (1): Descriptive statistics showed the frequency and percentage of gender, age and BMI for control and CAD obese patients

Variable	Cases	Control		CAD obese patients	
		Frequency	Percent	Frequency	Percent
Gender	Male	25	56.8	37	67.7
	Female	19	43.2	7	12.3
	Total	44	100.0	44	100.0
Age (year)	22 - 35	22	50.0	2	4.5
	>35	22	50.0	42	95.6
	Total	44	100.o	44	100.0
BMI	26 - 35	25	56.8	20	45.5
	> 35	19	43.2	24	54.5
	Total	44	100.0	44	100.0
Duration	≤ one year	-	-	5	11.4
	> one year	-	-	39	88.6
	Total	-	-	44	100.0

Results of PCR test

The extracted DNA obtained from the studied samples was genotyped using Polymerase Chain Reaction (PCR) and sequincing. The genotype assay was used for the analysis of the adiponectin gene (ADIPOQ) G276T for testing the hypothesis by analyzing the genotypes for single nucleotide polymorphism (G276T) in the ADIPOQ gene.

PCR – analysis

The PCR technique was used to amplify the regions that contain G276T for all collected samples and then homozygous and heterozygous genotype was found in each of the selected group (control and coronary artery cases) as shown in table2.The contingency table analysis (Crosstabs) was used to compute the homozygous and heterozygous

genotype distribution, odd ratio (OR) with 95% confidence interval (95% CI) and risk ratio (RR) (associated with patients) between the studied groups.

As shown in the table that out of 64 genotype assay (polymerase chain reaction (PCR) and sequencing assays for control monitoring,58(45.3%) were heterozygous corresponding to 6 (4.7%) homozygous, whereas for coronary artery patients 60 (46.9%) were heterozygous and 4 (3.1%) were homozygous genotypes. Statistical analysis revealed that the difference between the groups (control and coronary artery cases) was insignificant (df = 1, P – value = 0.510, odds ratio (OR) (95% CI) = 1.551, (0.416 – 5.783) and risk ratio (RR) (95% CI) = 0.787, (0.361 – 1.715).

Genotype distribution of the ADIPOQ gene G276T SNP

Table 3 shows the genotype frequencies of G276T SNP variant for control and coronary artery cases. The genotype frequencies of control group were 6 (4.7) normal (TT), 13 (10.2) AG, 4 (3.1) AT, 1 (0.8) CG, 8 (6.2) CT and 32 (25.0) GT, While for coronary artery patients were 4 (3.1) normal (TT), 13 (10.2) AG, 4 (3.1) AT, 3 (2.3) CG, 12 (9.4) CT and 28 (21.9) GT. The GG normal genotype was not observed in both groups. Odds ratio was not calculated because it is determined only for 2 X 2 tables.

Table (2): Analysis of the PCR product that contains G276T of the ADIPOQ gene for detection of homozygous and heterozygous genotype

Variable	Condition	Genotype frequency		Total
		Homozygote	Heterozygote	
Group	Control	6 (4.7)*	58 (45.3)	64 (50.0)
	coronary artery cases	4 (3.1)	60 (46.9)	64 (50)
	Total	10 (7.8)	118 (92.2)	128 (100)
Statistics	df	1		
	P – value	0.510		
	Sig. level	Ns		
	Odds ratio (95% CI)	1.551 (0.416 – 5.783)		
	Risk ratio (95% CI)	0.787 (0.361 – 1.715)		

ns: No significant difference

* Values in parenthesis represent the percentage from the total

Table (3): Genotype frequency of ADIPOQ gene G276T SNP

Variable	Condition	Genotype frequency							Total
		GG	AG	AT	CG	CT	GT	TT	
Group	Control	0 (0.0)*	13 (10.2)	4 (3.1)	1 (0.8)	8 (6.2)	32 (25.0)	6 (4.7)	64 (50.0)
	coronary artery cases	0 (0.0)	13 (10.2)	4 (3.1)	3 (2.3)	12 (9.4)	28 (21.9)	4 (3.1)	64 (50.0)
	Total	0 (0.0)	26 (20.4)	8 (6.2)	4 (3.1)	20 (15.6)	60 (46.9)	10 (7.8)	128 (100.0)
Statistics	Df	5							
	P – value	0.801							
	Sig. level	ns							

ns: No significant difference

* Values in parenthesis represent the percentage from the total

The difference between the two groups for genotype variant of G276T SNP was statistically insignificant (df = 5, P – value = 0.801> 0.050).

As shown from table 13 that the higher genotype frequencies (32 (25.0) and 28 (21.9)) were associated with the genotype GT for control and coronary artery patients, respectively. This was followed by AG, which achieved 13 (10.2) individuals for each group.

Genotypes location

Table 4 shows that for both control coronary artery patients 12 (92.3) of genotype AG were at the position rs15734 A>G and 1 (7.7) at rs15388 G>A (total 13), whereas for the genotype AT both groups showed 4 (75.0) at position rs15465 T>A and 1 (25.0) at rs15454 A>T. As for the genotype CG the position of the one heterozygous of control was at rs15424 G>C, while 2 (66.7) of patients were at rs15466 C>G and 1 (33.3) was at rs15424G>C. The genotype CT for control showed 5 (62.5) at the position rs15425 C>T and 1 (12.5) at the positions rs15426 C>T, rs15465 T>C and rs15465 T>C, respectively. For coronary artery patients this genotype (CT) showed 6 (50.0) at the position rs15425 C>T, 2 (16.7) at rs15423 C>T, 2 (16.7) at rs15466 C>T, 1 (8.3) at

rs15426 C>T and 1 (8.3) at rs15465 T>C. Furthermore, the genotype GT for control recorded 15 (46.9) at the position rs15661 G>T,12 (37.5) at the position rs15430 T>G, 2 (6.3) at rs15401 G>T, 1 (3.1) at rs15423 T>G and 1 (3.1) at 15428 G>T. For coronary artery cases 16 (57.1) were at rs15661 G>T, 11 (39.3) at rs15430 T>G and 1 (3.6) at rs15455 G>T. The homozygous TT genotype showed 6 (100.0) for control at the position rs15661G>T and 4 (100.0) for patients at the position rs15661 G>T as shown in table4.

Table (4): Location distribution of genotypes for control and coronary artery patients

Genotype	Control		Cases	
	Position	Frequency	Position	Frequency
AG	Het.15388 G>A	1 (7.7)	Het.15388 G>A	1 (7.7)
	Het.15734 A>G	12 (92.3)	Het.15734 A>G	12 (92.3)
	Total	13 (100.0)	Total	13 (100.0)
AT	Het.15454 A>T	1 (25.0)	Het.15454 A>T	1 (25.0)
	Het.15465 T>A	3 (75.0)	Het.15465 T>A	3 (75.0)
	Total	4 (100.0)	Total	4 (100.0)
CG	Het.15424 G>C	1 (100.0)	Het.15424 G>C	1 (33.3)
	-	-	Het.15466 C>G	2 (66.7)
	Total	1 (100.0)	Total	3 (100.0)
CT	-	-	Het.15423 C>T	2 (16.7)
	Het.15425 C>T	5 (62.5)	Het.15425 C>T	6 (50.0)
	Het.15426 C>T	1 (12.5)	Het.15426 C>T	1 (8.3)
	Het.15465 T>C	1 (12.5)	Het.15465 T>C	1 (8.3)
	het15466 T>C	1 (12.5)	Het.15466 C>T	2 (16.7)
	Total	8 (100.0)	Total	12 (100.0)
GT	Het.15401 G>T	2 (6.3)	Het.15430 T>G	11 (39.3)
	Het.15423 T>G	1 (3.1)	Het.15455 G>T	1 (3.6)
	Het.15428 G>T	1 (3.1)	Het.15661 G>T	16 (57.1)
	het15430 T>G	12 (37.5)	-	-

	Het.15455 G>T	1 (3.1)	-	-
	Het.15661 G>T	15 (46.9)	-	-
	Total	32 (100.0)	Total	28 (100.0)
TT	Homo.15661 G>T	6 (100.)	Homo.15661 G>T	4 (100.0)
	Total	6 (100.0)	Total	4 (100.0)

* Values in parenthesis represent the percentage from the total of each group

Association between the SNPs and circulating adiponectin concentration:

Table 5 shows that the level of adiponectin was not significantly different (P = 0.791) among the seven detected genotypes and it was ranged between 0.90±0.50 for TT genotype and 0.46±0.35 for CG genotype.

Table (5): Association between the SNPs and circulating adiponectin concentration.

Genotype	Adiponectin level
AG	0.78±0.35 [a]
GT	0.72±0.40 [a]
CT	0.77±0.53 [a]
CG	0.46±0.35 [a]
AT	0.89±0.33 [a]
TT	0.90±0.50 [a]
SE±	0.14
P – value	0.791

Means which followed by similar letters are not significantly different at 0.05 level of probability according to DMRT.

Discussion

Adiponectin is an adipokine that regulates lipid and glucose metabolism and has been shown to have anti-inflammatory and anti-atherogenic effects. It also plays an important role in the development of cardiovascular disease (CVD)[2].

In the present work statistical analysis revealed that the difference between the groups (control and coronary artery cases) concerning the type of Polymorphism (Homozygote & Heterozygote) was insignificant. This result was in agreement with the findings of (Sefa et al., 2017)[15], which found that Gene polymorphisms of adiponectin at rs1501299 and their allele frequencies were similar between the study and the control groups ($p>0.05$) while the same study found that adiponectin rs2241766 gene polymorphism and allele frequency were statistically significantly lower in the study group ($p<0.05$). this contrast may be due to different of genes studied by the current work which was G276T. same result also obtained by (Nasser,.et al 2012) [16] ,(Van et al, 2014)[17]and (Somayeh et al., 2016)[18].

Ohashi et al . examined the association of the Adiponectin gene polymorphisms with the incidence of coronary artery diseases in 383 Japanese patients with confirmed coronary artery diseases. On the other hand, Filippi et al recently reported that in 325 coronary artery disease patients and 270 controls, SNP+276 showed significant association with coronary artery disease. The reason for the discrepancies with the previous studies could be explained by the small sample size and these discrepancies could be partly explained by ethnic differences, which needs further research in larger numbers of study subjects.

Conclusion

Based on the findings of the present study, it could be concluded that:

Difference between the groups (control and coronary artery cases) concerning the type of Pholymorphysism (Homozygote & Heterozygote) was insignificant. While the level of adiponectin was not significantly different ($P = 0.791$) among the seven detected genotypes.

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