Research Article

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

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Abstract

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

The 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.

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.

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

Introduction:

Adiponectin is viewed as an insulin-sensitizing hormone with anti-inflammatory effects (Szmitko *et al*., 2013). 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 (Berg *et al*., 2015). Adiponectin has beneficial effects in the cardiovascular system by directly acting on the component cells of the heart and blood vessels. (Rowyda *al*., 2009). the associations between adiponectin gene in the ADIPOQ and CVD were significant according to (Huan *et al*.,2012).

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 unclear.(Kuo *et al*., 2014).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 originate from adipose tissue, especially adiponectin, have received growing attention.(Han *et al*., 2007).

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.

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.

Extraction of DNA from collected samples was performed using Salt Method which as follow:

A small amount from collected samples has been taken and shopped with a sterile scalped blade. 600 µl of TNES buffer was added to 35 µl of Proteinase-K (20 mg/ml), and the sample Incubated overnight (or 5-24 hours) at 50ºC. After that 166.7 µl of 6 M NaCl was added and the samples shacked vigorously for 20 seconds. Then, the samples centrifuged at full speed (10956rcf) for 5-10 minutes at room temperature. An equal volume (~ 800 µl) of cold 100 % ethanol added and gently mixed by inverting the tube a couple of times and white DNA precipitated out of solution. After that The DNA pellet was washed in 200-700 µl of 100 % ethanol. Then the ethanol poured off the ethanol and briefly.

Genomic DNA was extracted from ethylene diamine tetra acetic acid (EDTA)-whole blood samples by salt method. 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 and2.0µl 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 2% agarosegel (ROWYDA *et al*., 2012).

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 table13 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:

Table4 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:

Table5 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

In the present work statistical analysis revealed that the difference between the groups (control and coronary artery cases) concerning the type of Pholymorphysism (Homozygote & Heterozygote) was insignificant. This result was in agreement with the findings of (Sefa et al., 2017), 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), (Van et al, 2014) and (Somayeh et al., 2016)

Conclusion:

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

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

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