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Investigation of the relationship between adiponectin gene polymorphism (ADIPOQ SNP rs-266729) and obesity Patients / Basra-Iraqi

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Abstract

Adiponectin is one of the most important hormones secreted by adipose tissue and plays a major role in the development of obesity. A variant-specific ADIPOQ single nucleotide polymorphism (SNP rs-266729) with a C to G missense mutation can be strongly associated with obesity. This study aims to evaluate the association of single nucleotide polymorphisms (SNP rs-266729) of the ADIPOQ gene with obesity and some biochemical markers in obese patients. A case-control study included 186 participants (106 patients and 80 healthy control), the study work was carried out in a unit PCR lab in the Biology department / College of Education for the Pure Sciences / University of Basra. DNA was extracted from whole blood and then the Restriction Fragment Length Polymorphism Polymerase Chain Reaction (RFLP-PCR) technique was used to limit the genotype of the ADIPOQ gene (rs266729) polymorphism. Also, the lipid profile, glycated hemoglobin (HbA1C), fasting blood glucose (FBG), insulin, and adiponectin were measured by standard methods. Results: The allele G in the ADIPOQ-gene polymorphism SNP (rs266729) was a significantly higher frequency of 23 (21.6%) in the obese patients (P_Value = 0.039) compared with the control subject of 10 (12.5%). It significantly increased the risk in the additive model and allele frequency (P = 0.0139 and 0.0133), respectively. The rs266729 SNP showed a significant association with increased BMI, insulin, HOMO-IR, TG, VLDL, and lower adiponectin at a p-value of 0.001 for all, after adjustments for age and sex. Lower levels of high-density lipoprotein (HDL) were observed in the carriers CG and GG genotypes compared to the ADIPOQ rs266729 SNP C.C genotype (p = 0.032). In obese individuals, there was no significant relationship between rs266729 SNP and HbA1C, FBS, TC, and LDL. In conclusion, the single nucleotide polymorphism (rs266729) of the ADIPOQ gene has a significant difference with BMI, insulin, TG, VLDL, and HOMO-IR in obese patients, which might cause obesity.

Keywords: ADIPOQ gene polymorphism, BMI, Obesity, SNP (rs266729).

دراسة العلاقة بين تعدد الاشكال الجيني للاديبونكتين (ADIPIQ RS226729) و مرضى السمنة / البصرة-العراق

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الاديبونكتين احد اهم الهرمونات التي تفرز من الانسجة الدهنية والتي يلعب دور مهم في تطور السمنة. تعدد اشكال النيوكليتيدات المفردة (rs266729) لجين الاديبونكتين والطفرات الخاطئة من السايتوسين C الى الكوانين G قد يكون لها ارتباط قوي بالسمنة. هذة الدراسة تهدف الدراسة الى تقييم ارتباط تعدد اشكال النيوكليوتيدات المنفردة (SNP rs266729) في جين الاديبونكتين (ADIPO gene) مع السمنة وبعض المتغيرات البايوكيميائية في مرضى السمنة. شملت الدراسة ١٨٦ مشاركا (١٠٦ مريض بالسمنة و ٨٠ شخصا سليما). اجريت الدراسة في مختبر وحدة الPCR قسم علوم الحياة\ كلية التربية للعلوم الصرفة\ جامهة البصرة. تم استخلاص الحمض النووي من الدم الكلي للانسان، و استخدمت تقنية تفاعل البوليمراز المتسلسل Restriction Fragment Length Polymorphism Polymerase Chain Reaction (RFLP-PCR) لتحديد (SNP rs266729) . مجموعة الدهون، السكر التراكمي (HbA1C) ، السكر اليومي، هرمون الانسولين، و تركيز هرمون الاديبونكتين حيث تم قياسهم بواسطة طرق قياسية. في هذة الدراسة، كانت نسبة الاليل G (21.6%) 23 في (rs266729) عند مرضى السمنة و ذات فرق معنوي (p=0.039) عند المقارنة مع مجموعة السيطرة (12.5%) 10. وزيادة معنوية في اليل G عند additive model و allele frequency عند قيمة معنوية (P= 0.0139, 0.0133) علي التوالي. بين تعدد اشكال النيوكليوتيدات المفردة (SNP rs266729) علاقة معنوية مع مؤشر كتلة الجسم (BMI)، هرمون الانسولين Insulin، مقاومة الانسولين (HOMA-IR) , الدهون الثلاثية (TG), البروتين الدهني منخفض الكثافة (VLDL), وهرمون الاديبونكتين عند قيمة معنوية (P= 0.001) للكل, بعد استعاد العمر و الجنس. لوحظ انخفاض في مستوى الدهون عالية الكثافة (HDL) في حاملي الانماط الجينية GG و CG مقارنة مع النمط الجيني CC عند قيمة معنوية (P = 0.032). بينما لم تظهر فروق ذات دلالة احصائية بين (rs9939609 SNP) والسكر التراكمي A1C ،الدهون الكلية TC، السكر اليومي FBS، و LDL في مرضى السمنة. في الخلاصة،

فأن تعدد اشكال النيوكليتيدات المفردة (rs266729) لجين الاديبونكتين علاقة معنوية مع مؤشر كتلة الجسم (BMI)، هرمون الانسولين Insulin، الدهون الثلاثية (TG), مقاومة الانسولين (HOMA-IR) في مرضى السمنة، والتي قد تكون سبب السمنة.

الكلمات المفتاحية: تعدد الاشكال الجيني لجين الاديبونكتين، موشر كتلة الجسم، السمنة، و تعدد النتوكليوتيدات المفردة (rs266729).

Introduction

Adipose tissue is one of the important endocrine organs in the body; the main function of adipocytes is as energy storage depots and excretion of important hormones such as adiponectin and leptin [(1)].

Adiponectin is a 241 amino acid protein hormone encoded by the ADIPOQ gene on chromosome 3q27.3 that contains a signal region at the NH2-terminus, a variable region, a collagenous domain, and a globular domain at the COOH-terminus and is involved in regulating glucose levels and fatty acid breakdown [(2,3)], which is found in the proximal promoter region of the ADIPOQ gene. The ADIPOQ rs266729 polymorphism regulates adiponectin promoter activity as well as adiponectin levels. This ADIPOQ variation has been linked to insulin resistance and a high body mass index [(4,5)]. One of the most extensively researched indicators for T2D and obesity is the functional single nucleotide polymorphism (SNP) rs266729, which occurs in the promoter region of the ADIPOQ gene. It causes an amino acid shift from cytosine to guanine at nucleotide position -11,377 (-11,377 C>G) [(6)].

Adiponectin has many important functions in the body, the most important of which is increasing insulin sensitivity, with lower plasma concentrations associated with insulin resistance and fatty acid oxidation [(7,8)]. In addition, lower adiponectin protein levels are a risk factor for diabetes type 2 [(9)]. Therefore, in this research, we compared and analyzed the relationship between adiponectin gene polymorphism (rs266279) and some biochemical markers and the effect of this development on obesity and diabetes.

Subjects and Methods

Study subject

A case control study conducted between February 2022 to April 2023 comprised 186 participants who were divided into two groups: 106 patients with obesity who attended the Obesity Center at Al-Fayhaa Teaching Hospital in Basra City, and in addition, 80 completely healthy individuals as the control group. Participants in the study range in age from 18 to 45 years. The BMI for the patient group was greater than 30 kg/m^2, and the control group's BMI ranged from 18.5 - 24.9 kg/m2. Chronic disease (Diabetes), obesity due to secondary causes (hypothyroidism), pregnant women, and patients who take any treatment for obesity were excluded from the study.

Biochemical and genetic assessments

Fasting blood glucose (FBG), Total cholesterol (TC), Triglyceride (TG), Low-density lipoprotein cholesterol, and High-density lipoprotein cholesterol were determined with an automated chemical analyzer, the Cobas E311 device (Roche, Germany) [(10)]. Fasting plasma insulin and adiponectin levels were measured by the enzyme-linked immunosorbent assay (ELISA) and ELK Biotechnology (China). Glycated hemoglobin (HbA1c) was measured using a fully automated device (Program D-10 Hemoglobin A1c) [(11)]. Genomic DNA extraction from peripheral blood by using the Genomic DNA Mini Kit (Geneaid, Korea) [(12)], the concentration and purity of the genomic DNA were measured by UV absorption (Nano-drop, USA) at 260 and 280 nm. The total of the PCR mixture is 20µl containing 2 µl forward primer, 2µl reverse primer, 5 µl DNA sample, and 11µl deionized water nuclease-free. PCR amplification conditions were 4 min at 95 °C for denaturation followed by 35 cycles of 45 s at 94 °C to denature, 58 °C for 1min to anneal the primers and 72 °C for 5 minutes to final extension. The ADIPOQ gene genotyping was determined by using Restriction Fragment Length Polymorphism (RFLP-PCR). The primer sequences and PCR program in this study for the ADIPOQ gene SNP (rs266729) were obtained from Polska Tom et al. [(13)], as shown below.

Forward 5′-GGTGGACTTGACTTTACTGG-3

Revers 5′-TAGAAGCAGCCTGGAGAA-3

Statistical Analysis

MedClac-version 20.115 (https://www.medcalc.org /calc/odds_ratio.php). The Crosstabs test was used to analyze the genotypes and allele distribution in the control and patient populations. SPSS for Windows (version 26; USA). Student's t-test and ANOVA test were used to analyze the relationship between rs266729SNP in the adiponectin genotype group and biochemical markers.

Results Discussion

The obese patients consisted of 57 males and 49 females and the healthy control included 40 males and 40 females.

The biochemical and clinical characteristics of the study subjects were presented in Table 1. There were significant differences in the levels of BMI, FBS, insulin, and HOMA-IR in the obese patients when compared with the healthy group ($p = 0.02, 0.031, 0.013$, and 0.001, respectively). Also, were highly significant differences in the levels of TC, TG, LDL, and VLDL in obese patients when compared with

the control group, with (P_Value = 0.001) for all. Furthermore, the study noted a decreased level of HDL-C and adiponectin (P-value = 0.001) in obese patients when compared to the control group. There were no significant correlated (P-value > 0.05) in the HbA1c levels, sex, and age in the patients.

Table 1. Participants' anthropometric and biochemical features.

Variables	Control (N=80) Mean ± SD	Patients (N=106) Mean ± SD	P. Value
Age (years)	30.28 ± 5.87	30.62 ± 6.01	0.329
BMI (Kg/m2)	22.68 ± 2.14	39.54 ± 5.31	**0.024**
FBS (mg/dl)	98.6 ± 14.6	108.8 ± 22.1	**0.031**
HbA1C (%)	4.99 ± 0.56	5.11 ± 0.59	0.161
TC (mg/dl)	138.2 ± 20.7	184.5 ±37.6	**0.001**
TG (mg/dl)	110.7 ± 24.9	175.4 ±75.7	**0.001**
HDL (mg/dl)	32.65 ± 8.14	22.95 ± 5.87	**0.001**
LDL (mg/dl)	77.3 ± 19.03	111.3 ±32.9	**0.001**
VLDL (mg/dl)	73.83 ± 8.33	33.71±14.45	**0.001**
Insulin mIu/L	10.65 ± 5.38	57.6 ± 41.2	**0.013**
HOMA IR	2.68 ± 1.33	15.9 ± 11.8	**0.001**
Adiponectin (ng/ml)	9.86 ±3.26	8.15 ± 3.45	**0.001**

T-Test, Significant; P-value <0.05, SD: Standard division, FBS: Fasting blood sugar, HbA1C: glycated hemoglobin, TC: Total cholesterol, TG: triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very Low-density lipoprotein, HOMA IR: Homeostatic Model Assessment for Insulin Resistance.

Genotyping

As shown in "Figure. 1", the analysis of PCR genotypes of the ADIPOQ gene SNP (rs266729) by agarose gel electrophoresis discovered two bands (334, 212 bp) for CC wild-type, three bands (334, 212, 122 bp) for CG heterozygous genotypes, and two bands (334, 122 bp) for GG homozygous genotypes, E. Polska Tom et al., [13].

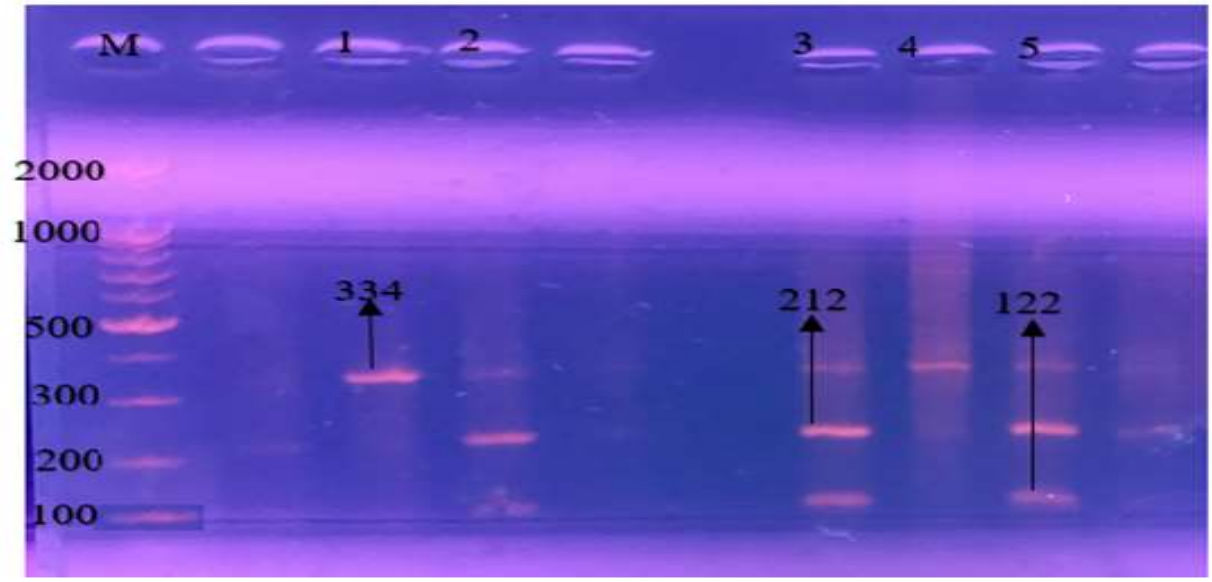

Figure 1. RFLP-PCR product of ADIPOQ gene SNP (rs266729) on agarose gel electrophoresis (2%, 100V for 60 minutes), M: Marker line (100bp). ADIPOQ genotypes: lines 1,2,3,4,5.

Table 2 showed the genotypes' compatibility with Hardy–Weinberg equilibrium in obese and the control individuals' P- value equal to (0.0816) and (0.127), respectively.

Table 2. Shown Hardy-Weinberg equilibrium for the ADIPOQ gene SNP (rs266729) in patients and control.

Subjects	X^2	P. Value
Patients	5.0109	0.0816
Controls	4.124	0.127

X^2= Chi-Square

The allele frequencies and genotypes of the ADIPOQ gene variants were shown in "Table 3". No statistically notable differences correlation was identified between the GG and CG genotypes of the ADIPOQ gene variant rs266729 in obese and control persons. while the CC genotype showed a statistically significant difference. In the co-dominant model, the frequencies of GG in the control were 10 (12.5%) and patient 23 (21.6%), and the frequencies of CG in the control were 25 (31.25%) and patient 40 (37.7%); OR = 1.9398 and 1.3333, respectively. There are no notable differences were shown between GG and CG genotype distributions among control and patient populations (P. Value = 0.107, 0.3589 respectively). The CC genotypes were significantly higher (P = 0.034) in the patients' 43 (40.5%) compared with the control's 45 (56.25%), OR = 0.5309.

In the dominant model, there were highly significant statistical differences in GG+CG genotype frequencies in patients 63 (59.4%) and controls 35 (42.6%), OR = 1.8837, and P-value 0.0347. In the recessive model, the CC+CG genotype was found at 70 (87.5%) and the GG genotype was found at 10 (12.5%) in the control group, which was not significant (P-value 0.1079) compared to 83 (78.3%) and 23 (21.6%) in obese patients for CC+CG and GG, respectively.

In the additive model, allele G frequency shows a significant difference (p = 0.013 and 0.0133, respectively). The 2GG+CG genotype and G allele frequency were found in 45 (56.25%) of the control patients, which was less than the patient population of 86 (81.1%). In the additive model, the odd ratio for 2GG+CG was (2.000), while the odd ratio for the G allele was (1.744).

Table 3. Summarizes the genotype and allele frequency of the ADIPOQ gene SNP (rs266729) in patients and controls.

Rs9939609	Controls N=80	Patients N= 106	OR (95% CI)	Adjusted OR (95% CI)	P_ Value
Codominant					
CC	45 (56.25%)	43(40.5%)	0.5309	0.2949 to 0.9555	**0.034**
CG	25 (31.25%)	40 (37.7%)	1.3333	0.7211 to 2.4653	0.3589
GG	10 (12.5%)	23 (21.6%)	1.9398	0.8649 to 4.3503	0.107
Dominant					
GG+CG	35 (42.6%)	63 (59.4%)	1.8837	1.0466 to 3.3906	**0.0347**
Recessive					
CC+CG	70 (87.5%)	83 (78.3)			
GG	10 (12.5%%)	23 (21.6%)	0.5155	0.2299 to 1.1562	0.1079
Additive					
2(GG)+CG	45 (56.25%)	86 (81.1%)	2.0000	1.1515 to 3.4736	**0.0139**
Frequency of G allele	45 (56.25%)	86 (81.1%)	1.7443	1.1229 to 2.7094	**0.0133**

Crosstabs-test (Fisher exact test).

The BMI, FBS, HbA1C, TC, TG, VLDL, HDL, LDL, fasting insulin, and HOMA-IR of patients with the ADIPOQ gene (rs266729) in the codominant model were analyzed by ANOVA "Table 4". There was revealed no significant variance among the CC, GG, and C.G genotypes of ADIPOQ rs266729 SNP; FBS, TC, LDL, and HbA1C (P-Value > 0.05) among genotypes of patients. There were highly significant differences in BMI, insulin, HOMA-IR, TG, VLDL, and adiponectin levels between the three genotypes (P-value = 0.001). This study showed significant variance in the serum levels of HDL with three different genotypes (CC, G.G, and CG) at P = 0.032.

Table 4. Shows the clinical features of patients according to the genotypes of the ADIPOQ gene SNP (rs266729) (co-dominant model).

Variables	CC (n=43)	CG (n=40)	GG (n=23)	P. Value
BMI (kg /m ^{2})	36.41 ± 4.04	38.31 ± 4.82	40.25 ± 6.02	**0.001**
FBS (mg/dl)	103.24±15.09	103.34±18.21	107.49 ± 17.76	0.472
HbA1C %	4.98 ± 0.561	5.03 ± 0.481	5.11 ± 0.66	0.609

Continued Table 4.

Insulin (μU/ml)	29.91 ± 23.68	56.02 ± 29.23	111.52 ± 34.29	**0.001**
HOMA-IR	11.44 ± 10.77	15.24± 10.24	24.24 ± 15.45	**0.001**
Cholesterol (mg/dl)	174.77 ± 30.27	186.95 ± 46.29	189.44 ± 40.5	0.191
Triglycerides (mg/dl)	148.31 ± 68.06	163.60 ± 67.51	246.64 ± 59.22	**0.001**
VLDL (mg/dl)	30.06 ± 14.87	32.82 ± 13.06	49.44 ± 11.86	**0.001**
LDL (mg/dl)	102.67 ± 36.24	107.83 ± 27.28	119.5 ± 33.69	0.109
HDL (mg/dl)	35.27 ± 10.12	30.27 ± 7.15	27.78 ± 6.71	**0.032**
Adiponectin (ng/ml)	11.09 ± 3.15	6.82 ± 2.12	4.98 ± 1.12	**0.001**

One-Way ANOVA test

Discussion

This study is the first in Iraq to assess whether the adiponectin variant rs226729 SNP is correlated with weight gain and body mass index. This work analyzed the ADIPOQ gene of the SNP rs266729 in a group of normal-weight and obese Iraqi patients and the association between the ADIPOQ gene polymorphism with some metabolic biochemical markers. Also, the role of the ADIPOQ gene in obesity and the risk for diabetes mellitus (T2D) and heart disease [14,15].

The current study found that the adiponectin variant rs226729 SNP is significantly connected with elevated levels of the insulin hormone, HOMO-IR, and BMI, so the relationship of rs266729 with obesity and diabetes seems clear [16,17]. Obese patients with risk allele G of the ADIPOQ gene SNP rs226729 have been shown in the study to be significantly associated with higher BMI, HOMO-IR, TG, VLDL and insulin, these findings were in agreement with Na, *et al* 2022 and Cheng, Jin, *et al* 2021 [18,19].

Also, the study discovered a link between the ADIPOQ rs226729 polymorphisms and HDL-C levels and adiponectin. Carriers of the rs266729 polymorphism's allele G exhibited lower levels of HDL-c and adiponectin compared to allele C carriers, who had higher levels of HDL and adiponectin. These findings were similar to de Luis *et al*., 2020 [20].

Obese patients with homozygous GG genotype have higher significant serum levels of plasma insulin, TG, VLDL, and HOMO-IR compared with CC and CG genotypes which agreed with H. F. Gu., 2021[21]. While the low level of HDL in the homozygous GG genotype is compared with CC and CG genotypes [20].

Recent studies were shown that the allele G changes the sequence of one transcriptional stimulatory protein binding site and adiponectin-stimulatory activity in both white and brown adipose tissue [5,22]. In addition, many studies have shown the association between adiponectin levels and adipose tissue mass, as the levels of the hormone adiponectin in the blood circulation are inversely proportional to the fat mass, adiponectin levels decrease in people who suffer from obesity, and this lack of the hormone promotes the development of insulin resistance (IR) by preventing the phosphorylation of insulin receptors, and increasing the flow of fatty acids in the liver and slowing down their oxidation, and thus increasing the production of glucose and triglycerides [23,24].

The biochemical markers in this study showed that obesity had an effect on lipid and lipoprotein metabolism, and this is a risk factor for cardiovascular diseases by increasing the levels of Total-cholesterol, triglycerides, and low_ density lipoprotein, while the level of high-density lipoprotein (good lipoprotein) decreased in obese patients. These results agreed with those obtained by Sitar-Tăut *et al*., 2021 [25,26]. An increase in TG leads to a decrease in lipoprotein metabolism, especially HDL-C, which works to protect the walls of blood vessels and removed total cholesterol [27] .

In obese patients, the biochemical analysis revealed increased levels of insulin hormone, TG, VLDL, and HOMO-IR, as well as decreased adiponectin levels which leads to insulin-resistance (a lack of tissue response to the action of the insulin), which is leading cause T2DM and increased triglycerides in the liver [28]**.** Insulin resistance leads to impaired activity of endothelial-bound lipoprotein lipase (LPL), which is involved in reduced triglyceride hydrolysis and uptake of VLDL and chylomicron by adipose tissue and muscle [29,30].

Conclusion

The GG genotype of the ADIPOQ gene variant (rs266729) is connected with decreased adiponectin levels and increased TG, insulin, and HOMA_IR levels. Furthermore, the carriers of the C allele exhibited lower levels of adiponectin than carriers of the C allele and represented a risk factor for obesity.

Conflicts of Interest

The authors have no conflict of interest

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Ethics Statements

All locations where samples were obtained were given administrative approval, and all research participants gave their written and/or verbal consent**.**

Author Contribution

Fatima S. Sabah and Hamad J. Abas contributed to designing the study, the supervising this manuscript and analyzed the data. All authors wrote the paper, read and approved the final manuscript.

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