Research Article

The association of ghrelin -501A/C polymorphism with ghrelin and leptin levels in non-obese Saudi Population with type 2 diabetes mellitus

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Abstract

Background: Saudi Arabia is one of the three of the world's top 10 countries with the highest prevalence of diabetes mellitus. Ghrelin is a gut–brain endogenous peptide, and the genetic variations within the gene have been associated with the risk of developing type 2 diabetes mellitus (T2DM). **Aims and Objective:** To study the association of ghrelin -501A/C polymorphism with ghrelin and leptin levels in non-obese Saudi population with T2DM. **Materials and Methods:** Eighty unrelated Saudi subjects with diabetes and 56 healthy controls were recruited. Single nucleopeptide polymorphism (SNP) -501A/C (rs26802) of the ghrelin gene was genotyped by restriction fragment length polymorphism. Individuals were phenotypically characterized by body mass index, lipids, glucose, blood pressure, and leptin and ghrelin levels. **Results:** No significant difference in the -501A/C genotype distributions and allele frequency was observed between T2DM and control subjects (both $P > 0.05$). Plasma ghrelin was negatively correlated with serum glucose, triglycerides, and total cholesterol in Saudi patients with diabetes. However, in control persons, no significant correlation was observed. In T2DM group, the 501A/A and 501A/C genotypes were associated significantly with lower plasma levels of ghrelin compared with C/C mutant homozygotes ($P = 0.031$), while the polymorphism was not associated with the lipid profile, leptin levels, or blood pressure. **Conclusions:** Although, the plasma levels of ghrelin were lower in A carriers compared with C/C mutant homozygotes and A carriers were associated with lower ghrelin levels, the ghrelin gene -501A/C polymorphism has no significant relationship with the susceptibility of T2DM in the Saudi patients with diabetes.

KEY WORDS: Single Nucleopeptide Polymorphism; Ghrelin Gene; Diabetes Mellitus; Saudi Population

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Introduction

Diabetes mellitus (DM) is a chronic debilitating condition that is rapidly increasing in prevalence worldwide and three of the world's top 10 countries with the highest prevalence of diabetes are Saudi Arabia, Kuwait, and Qatar.[1]

Ghrelin is a 28-amino acid gut–brain endogenous peptide ligand for the growth hormone secretagogue receptor (GSH-R) secreted by the X/A-like cells in the stomach.[2] Ghrelin is a multifunctional peptide participating in growth hormone release, appetite regulation, and gut motility. In addition to

food intake and energy balance, ghrelin also controls glucose metabolism, and it has been shown that, in different pathophysiological conditions such as obesity, type 2 DM (T2DM), and other conditions with metabolic disturbances, ghrelin concentrations decrease.[3–6]

Genes and lifestyle interact in the development of T2DM. In persons with T2DM, the fasting ghrelin level is lower in obese than in lean persons, and its level is decreased in healthy offspring of patients with T2DM, which indicates the role of a possible genetic component in the regulation of ghrelin plasma levels.[7,8] Several polymorphisms of the ghrelin gene have been identified and studied over the last years in relation to control of satiety, obesity, eating behaviors, metabolic syndrome, glucose homeostasis, and T2DM.[9]

The significance of genetic variants of the ghrelin–ghrelin receptor axis in determining the glucose metabolism has been the objective of numerous small and larger studies. The results of these studies imply that the common genetic variants show either lacking or modest effects.[10] Ghrelin (GHRL) gene was, in one of the genetic association analyses, polymorphisms in 12 genes in the Finnish Diabetes Prevention Study (DPS), suggested to be associated with the risk of developing T2DM.[11]

Previous studies revealed that polymorphisms found in the coding region of the preproghrelin were responsible for causing obesity and might affect glucose-induced insulin secretion.[12] Cycle sequencing of genomic DNA revealed 11 single nucleopeptide polymorphisms (SNPs) in the 5′ flanking region of the ghrelin gene, and different mutations were found; one of these is the cytosine (C) to adenine (A) transitions at base 501, A to C at -501 (-501A/C); 501 is located in intron number one.[12]

Unfortunately, there is a gap in knowledge of the current situation regarding the ghrelin gene's -501A/C polymorphism and its association with T2DM in Saudi patients with diabetes in Taif City. To the best of our knowledge, there is no studies that examined and documented the magnitude and association of the ghrelin gene's -501A/C polymorphism and its association with T2DM in Saudi patients with diabetes in the study area. Our previous study[13] focused on identifying of the ghrelin gene's -501A/C polymorphism and the association of its different genotypes with obesity parameters and the relationship of these genotypes with ghrelin levels in the obese nondiabetic individuals living in Taif City.

The aim of the present study was to investigate the association of the ghrelin gene's -501A/C polymorphism with ghrelin and leptin levels, blood pressure, and lipid profile in non-obese Saudi population with T2DM, in Taif city, KSA.

Materials and Methods

Study Design and Population

A case–control study enrolled 80 unrelated subjects with diabetes recruited consecutively from those treated and followed up in the diabetic outpatient clinic in King Abdul-Aziz Hospital Taif, KSA, during the period from January to June 2014. A control group, of the same nationality (Saudi), aged 52 ± 4 years, same sex, non-obese [body mass index (BMI), 22–26 kg/m^2], with normal glucose levels, and without any other clinical components were recruited out of the orthopedic outpatient clinic' visitors in King Abdul-Aziz Hospital Taif, KSA.

Detailed medical histories of all the participants were obtained, and their systemic examinations were carried out by specialists. Participants who had a history of metabolic diseases, digestive, eating disorders, endocrine, or any other psychiatric disease were excluded from the study.

Approval for this study was obtained and granted by University of Taif. It also approved by Taif Directorate of Health, Ministry of Health, KSA. Informed verbal consent was secured from study participants in their own Arabic language after explaining the purpose of the study, potential risks and benefits of participating in the study, and the right to withdraw from the study at any time. The participants were also assured about the confidentiality of the data.

Leptin and Ghrelin Levels Assay

Fasting serum leptin levels were determined for the two groups using a commercially available leptin ELISA kit (DRG International, USA). Each sample was run in duplicates within the same plate, and a coefficient of variation was calculated. The average of the two leptin measurements was used for this analysis. Plasma ghrelin concentrations were measured using ELISA kits (DRG International).

Genotyping of the Ghrelin Gene Variants

SNP 501A/C (rs26802) in the promoter ghrelin gene was studied by restriction fragment length polymorphism (RFLP). Amplification of the DNA fragment containing the SNP was done by PCR as described by Vartiainen et al.[12]; then, the PCR product was digested with 0.025 U *Mwo*I restriction enzyme (New England Biolabs). After that, the mixture was incubated at 60°C for 16 h. The digestion of the DNA fragment consisting of 509 base pairs (bp) resulted in 136- and 372-bp fragments in the 501A/A genotypes, and in the 501C/C genotype, the 372-bp fragment was further digested into 241- and 141 bp fragments. Table 1 shows the primers used in this study. After electrophoresis on a 2% agarose gel (QA agar) containing 2 ng/mL ethidium bromide, the digestion fragments were separated and visualized on an ultraviolet transluminator.[12]

Sampling

Five milliliters of antecubital venous blood was aseptically collected in EDTA tube for PCR and genotyping and for assessment of leptin

Table 1: Locations and primer sequences of SNP analyzed

Variants	Locations	Primers
A-501C (rs26802)	Promoter	F: 5′-agaacaaacgccagtcatcc-3′ R: 5′-gtcttccagccagacagtcc-3′

SNPs, single nucleotide polymorphisms; F, R: forward and reverse primer sequences.

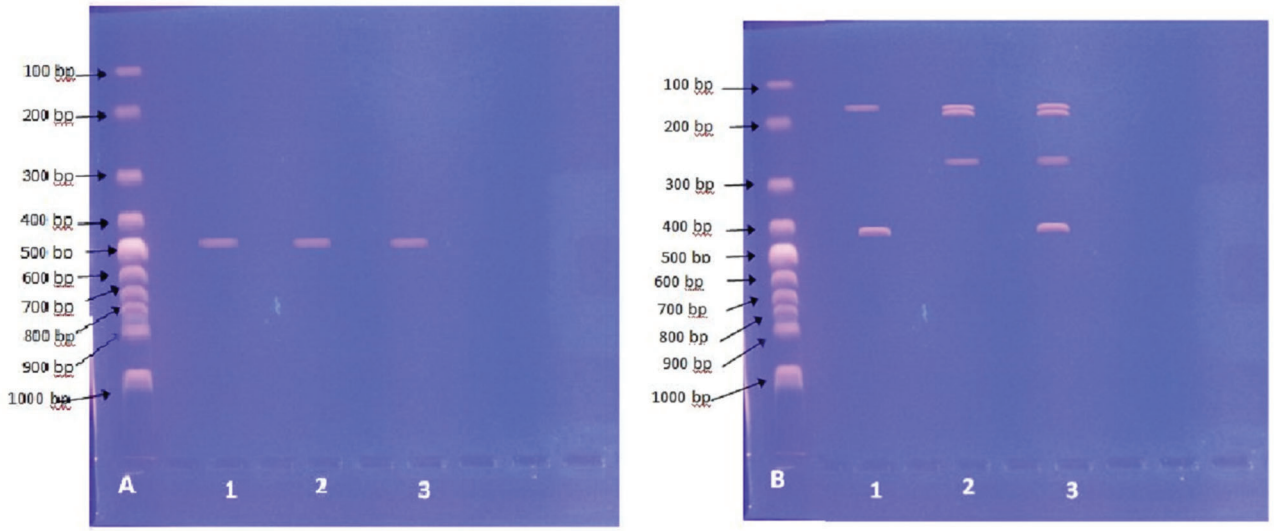

Figure 1: (A) The rs26802 gene segment without digestion; (B) Different gene fragments after digestion by *Mwo*I enzyme: lane 1, A/A genotype; lane 2, C/C genotype; lane 3, A/C genotype.

and ghrelin levels in plasma after 12- to 14-h fasting under complete aseptic precautions in plain test tubes without anticoagulant. After coagulation, samples were centrifuged (at 1500 × g for 15 min). The separated serum used for the immediate assay of fasting glucose and lipid profile. Repeated freezing and thawing were avoided. Hemolyzed samples were discarded.

Analytical Methods

Serum glucose level, total cholesterol (T-CHOL), triglycerides (TG), and high-density lipoprotein (HDL) were analyzed using Synchron CX?9 (Instruments, Inc., Scienfitic Instruments Division, Fillertron, CA) system autoanalyzer applying enzymatic colorimetric method.[14–17] Low-density lipoprotein (LDL) cholesterol was calculated according to "Friedewald equation," provided that the serum TG level is < 400 mg/dL[18]: LDL = Total cholesterol - (HDL + TG/5).

Body Mass Index

Body weight and height were measured, and BMI was calculated as weight (kg) divided by height squared(m^2) (kilograms per square meter).

Measurement of Blood Pressure

Blood pressure was measured twice from the right arm using a standard sphygmomanometer after 10 min of rest with the persons in a sitting position. The mean of the two measurements was used in the calculations.

Diagnosis of T2DM

Diagnosis of diabetes was according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus in 1997 and the follow-up reports in 2003.[19] We recognized T2DM by one of the following criteria: (a) symptoms of diabetes mellitus plus random blood sugar concentration ⩾200 mg/dL; (b) fasting blood sugar (FBS) ⩾ 126 mg/dL on more than one occasion; (c) 2-h postprandial plasma glucose concentration ⩾200 mg/dL during the oral glucose tolerance test.

Statistical Analysis

Data were expressed as the mean ± SD. Each variable was assessed for a normal distribution using the Kolmogorov–Smirnov test. Statistical differences between the groups were identified using one-way analysis of variance (ANOVA). Simple linear regression analysis with the genotype as a dependent variable was performed to identify its relation to ghrelin level. All statistical results were based on the two-sided tests. Data were analyzed using Statistical Package for Social Sciences (SPSS) software for Windows (version 22.0; SPSS, Inc., Chicago, IL). $P < 0.05$ was regarded as significant. Pearson correlation coefficient was used to explore the association between total ghrelin, leptin, and the metabolic and clinical variables.

Results

A case–control study including 80 Saudi subjects with diabetes and 56 age- and sex-matched control subjects was performed and genotyped for restriction fragment length polymorphism SNP -501A/C of the 5′ flanking region of the ghrelin gene by sequencing 136 DNA samples, and three genotypes were observed: -501A/A, -501A/C, and -501C/C (Figure 1A,B). Genotypes distributions and allele frequencies of the SNP -501A/C genotypes of the 5′ flanking region of ghrelin gene in the two study groups are shown in Table 2. There were no significant differences regarding genotype distributions and allele frequencies for the A-501C polymorphism between the

Table 2: Genotype distributions and allele frequencies of SNPs analyzed in the control group and patients with diabetes

Variants	Genotypes/alleles	Control, *N* (%)	Patients with diabetes, *N* (%)	*P**	OR (95% CI)
A-501C (rs26802)	Genotypes				
	AA	32 (57.14)	36 (45.00)	0.434, NS	0.61 (0.31–1.22)
	AC	16 (28.57)	36 (45.00)		2.0 (0.98–4.300
	CC	8 (14.29)	8 (10.00)	0.66 (0.22–1.97)	
	Alleles				
	A	80 (71.43)	108 (67.5)	0.290, NS	0.83 (0.49–1.41)
	C	32 (28.57)	52 (32.5)		

SNPs, single nucleotide polymorphisms; NS, not significant.
*P values of χ^2 test.

Table 3: Demographic and clinical characteristics of patients and controls

Variables	Control (n = 56)	Patients (n = 80)
Age (years)	55.49 ± 8.94	56.52 ± 2.35 NS
BMI(kg/m^2)	26.6 ± 3.2	26.85 ± 2.1 NS
SBP (mmHg)	119.5. ± 11.2	132.1 ± 23.7***
DBP (mmHg)	77.9 ± 6.8	79.5 ± 12.8***
FBS (mg/dl)	94.43 ± 10.06	130.17 ± 5.43***
TG (mg/dl)	93.66 ± 16.78	215.68 ± 21.80***
HDL-C (mg/dl)	42.53 ± 2.16	34.05 ± 5.19***
T-CHOL (mg/dl)	179.10 ± 16.96	199.17 ± 32.64***
LDL(mg/dl)	111.23 ± 11.42	146.74 ± 25.29***
Ghrelin (pg/dl)	719.93 ± 74.30	195.05 ± 50.57***
Leptin (ng/dl)	6.86 ± 2.8	22.00 ± 14.94***

***All values are arithmetic mean ± SD. Patients indicate subjects with diabetes; controls, subjects without diabetes; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; TG serum triglyceride; HDL-C, serum high-density lipoprotein cholesterol; T-CHOL, serum total cholesterol. The p-value is that of Student's t-test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, NS = not significant, patients with diabetes vs controls.

two groups (Table 2). Although patients with diabetes demonstrated a higher AC genotype distribution double times than control group [OR (95% CI): 2.0 (0.98–4.30)], the genotype distributions of this SNP followed the Hardy–Weinberg equilibrium in both the groups was not significant statistically ($P > 0.05$ for both). Table 3 shows demographic and clinical characteristics of the studied cases and control groups; no significant differences were found between the two groups regarding age and BMI. The group of patients with diabetes showed significantly higher systolic blood pressure (SBP), diastolic blood pressure (DBP), and FBS compared with the control group ($P < 0.001$). As regard lipid profile, there was a significant increase of serum TG and T-CHOL ($P < 0.000$), with a significant decrease in the level of HDL cholesterol in cases ($P < 0.001$) compared with control group. Ghrelin significantly decreased in the patients with diabetes compared with the control group ($P < 0.001$), while leptin was significantly higher in the patients with diabetes compared with the control group. Table 4 describes the main phenotype characteristics of the -501A/C genotypes of the ghrelin's gene in control subjects; no significant differences were observed in the studied parameters

Table 4: The main phenotype characteristics of the SNP -501A/C genotypes of the 5′ flanking region of ghrelin gene in control subjects

	Genotype-501A/C				Combined genotype-501A/C		
	A/A	A/C	C/C	*p*	A/A + A/C	C/C	*p*
Age (years)	57.52 ± 7.15	56.52 ± 7.32	55.52 ± 8.15	0.2342	57.02 ± 7.24	55.52 ± 8.15	0.262
BMI (kg/m^2)	27.07 ± 1.11	26.12 ± 1.2	26.01 ± 5.11	0.067	26.60 ± 1.16	26.01 ± 5.11	0.322
SBP (mmHg)	120.1 ± 2.4	117.1 ± 7.2	118.1 ± 4.3	0.072	118.60 ± 4.80	118.1 ± 4.3	0.534
DBP (mmHg)	78.5 ± 5.5	77.5 ± 4.5	79.5 ± 7.8	0.051	78.00 ± 5.00	79.5 ± 7.8	0.174
FBS (mg/dl)	94.00 ± 14.73	86.75 ± 45.04	94.75 ± 18.24	0.058	90.23 ± 40.04	94.75 ± 18.24	0.432
TG (mg/dl)	104.33 ± 8.32	91.15 ± 16.02	101.55 ± 18.8	0.943	106.34 ± 29.55	101.55 ± 18.8	0.607
HDL-C (mg/dl)	48 ± 4.28	52.75 ± 7.28	51.95 ± 7.60	0.406	52.91 ± 5.78	51.95 ± 7.60	0.404
T-CHOL (mg/dl)	171.07 ± 34.17	175.57 ± 15.11	176.07 ± 34.07	0.439	169.57 ± 24.29	176.07 ± 34.07	0.196
LDL-C (mg/dl)	107.33 ± 15.53	109.69 ± 10.31	110.11 ± 13.1	0.657	108.51 ± 12.92	110.11 ± 13.1	0.631
Ghrelin (pg/dl)	735.11 ± 38.16	701.44 ± 50.60	711.50 ± 75.59	0.052	718.28 ± 44.38	711.50 ± 75.59	0.513
Leptin (ng/dl)	7.70 ± 7.25	8.20 ± 12.04	8.08 ± 7.50	0.604	7.95 ± 6.15	8.08 ± 7.50	0.912

All values are arithmetic *mean?* SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; TG, triglycerides; HDL-C, high-density lipoprotein; T-CHOL, total cholesterol. p-Values are probabilities for the difference between the genotypes in ANOVA (Genotype -501A/C**)** or in independent t-test(combined genotype -501A/C**)**. p-Value <0.05 is considered statistically significant.

Table 5: The main phenotype characteristics of the SNP -501A/C genotypes of the 5′ flanking region of ghrelin gene in patients with diabetes

	Genotype -501A/C				Combined genotype -501A/C		
	A/A	A/C	C/C	*p*	A/A + A/C	C/C	*p*
Age (years)	53.52 ± 8.15	56.52 ± 5.36	55.52 ± 8.15	0.188	55.02 ± 6.755	55.52 ± 8.15	0.846
BMI (kg/m^2)	28.87 ± 1.11	26.12 ± 1.2	27.01 ± 1.11	0.087	29.095 ± 1.155	27.01 ± 1.11	**0.009**
SBP (mmHg)	132.1 ± 5.1	133.1 ± 4.2	130.1 ± 3.3	0.227	132.6 ± 20.7	130.1 ± 3.3	0.749
DBP (mmHg)	77.5 ± 10.5	79.5 ± 11.5	77.5 ± 12.8	0.751	78.5 ± 7	77.5 ± 12.8	0.728
FBS (mg/dl)	132.00 ± 1.41	129.90 ± 5.58	131.50 ± 4.94	0.951	135.25 ± 15.04	131.50 ± 4.94	0.930
TG (mg/dl)	215.00 ± 29.04	207.03 ± 17.92	211.17 ± 2.82	0.943	231.57 ± 174.56	211.17 ± 2.82	0.749
HDL-C (mg/dl)	32.5 ± 2.28	36.35 ± 3.28	35.05 ± 3.20	0.686	36.95 ± 9.28	35.05 ± 3.20	0.557
T-CHOL (mg/dl)	222.07 ± 5.17	218.07 ± 4.41	216.07 ± 6.07	0.739	211.07 ± 34.29	216.07 ± 6.07	0.689
LDL-C (mg/dl)	189 ± 4.24	181.35 ± 18.29	192.50 ± 6.36	0.673	188.50 ± 9.96	192.50 ± 6.36	0.582
Ghrelin (pg/dl)	185.11 ± 48.86	201.44 ± 50.65	211.00 ± 55.59	0.154	185.11 ± 49.755	211.00 ± 55.59	**0.031**
Leptin (ng/dl)	21.00 ± 17.26	23.00 ± 12.04	22.00 ± 17.10	0.854	21 ± 14.65	22.00 ± 17.10	0.857

All values are arithmetic mean??SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; TG, triglycerides; HDL-C, high-density lipoprotein; T-CHOL, total cholesterol. *p*-Values are probabilities for the difference between the genotypes in ANOVA (Genotype-501A/C**)** or in independent *t*-test (combined genotype-501A/C). *p*-Value <0.05 is considered statistically significant. Bold *p*-values are significant.

Table 6: Pearson correlation coefficient (*r*) between ghrelin and leptin and the measured parameters in control group and patients with diabetes

	Ghrelin		Leptin	
	Patients	Control	Patients	Control
SBP (mm Hg)	-0.577*	-0.277	0.432*	0.531*
DBP (mm Hg)	-0.553	-0.351	0.376*	0.176
FBS (mg/dL)	-0.502	-0.302	0.591*	0.294
TG (mg/dL)	-0.553*	-0.253	0.496	0.386*
HDL-C (mg/dL)	0.376*	0.178	-0.365*	-0.265
T-CHOL (mg/dL)	-0.603*	-0.214	0.479*	0.512*

SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; TG, triglycerides; HDL-C, high-density lipoprotein; T-CHOL, total cholesterol.
**Significant $P < 0.05$.

between different genotypes. In addition, Table 5 describes the main phenotype characteristics of the -501A/C genotypes of the ghrelin's gene in patients with diabetes. Regarding BMI, no statistically significant difference between the three genotypes in patients with diabetes was found ($P = 0.087$), but when A/A and A/C carriers were combined (A carriers) and compared with C/C mutant homozygotes, a statistically significant difference in the mean BMIs between the SNP-501A/C genotypes ($P = 0.009$) was observed. No differences in the plasma levels of leptin between the genotypes were observed ($P = 0.857$). Regarding blood pressure, lipid profile, and FBS, all showed no significant differences between the genotypes even when the genotypes were combined ($P > 0.05$). Moreover, plasma levels of ghrelin were lower in A carriers compared with C/C mutant homozygotes ($P = 0.031$). Table 6 shows, in the cases group, a significant negative correlation between plasma ghrelin and SBP ($r = -0.577$), DBP ($r = -0.553$), FBS ($r = -0.502$), TG ($r = -0.553$), and T-CHOL ($r = -0.603$). On the other hand, leptin revealed a significant

Table 7: Coefficients of regression analysis to determine the relationship between ghrelin level and different ghrelin gene SNP genotypes in control group and patients with diabetes

	Control group				Patients with diabetes			
	Beta	Confidence level		*P*	Beta	Confidence level		*P*
AA	0.45	-40.12	45.65	0.565, NS	-1.985	-87.12	75.65	0.040, Sig
AC	0.343	7.89	47.2	0.059 NS	-1.673	-8.86	37.2	0.042, Sig
CC	-0.432	-48.43	-29.1	0.090 NS	-0.032	-40.43	-27.1	0.190, NS

AA, A/A genotype; AC, A/C genotype; CC, C/C genotype; NS, not significant; Sig, significant.

positive correlation with them (r = 0.432, 0.376, 0.591, 0.496, and 0.479, respectively). However, plasma ghrelin showed a significant positive correlation with HDL cholesterol (r = -0.376), whereas leptin showed a significant negative correlation with HDL (r = -0.365). Table 6 also revealed, in the control group, no significant correlation between ghrelin and the studied parameters; however, leptin correlated positively with the SBP, TG, and T-CHOL (r = 0.531, 0.386, and 0.512, respectively). Table 7 revealed that AA and AC genotypes (A carriers) are associated with lower ghrelin levels (P = 0.040 and 0.042, respectively).

Discussion

The effects of ghrelin gene polymorphism is a complex area of investigation, owing to ghrelin's interplay with a host of various factors.[9] In this study, we focused on one of the SNPs in the ghrelin gene 5′ flanking area, the SNP -501A/C in patients with diabetes. Ghrelin significantly decreased in the patients with diabetes compared with control group, while leptin was significantly higher in the patients with diabetes compared with control group. Preceding studies on ghrelin revealed that ghrelin levels were lower in patients with T2DM when compared with normal persons.[20] In obese patients with T2DM, plasma levels of ghrelin were significantly lower compared with non-obese patients.[21] Our results are inconsistent with a previous study,[22] where decreased plasma levels of active ghrelin were significantly associated with abdominal adiposity. The presence of a significant negative correlation between plasma ghrelin and SBP, DBP, FBS, TG, and T-CHOL in patients with diabetes in this study revealed the association of hypertension, insulin resistance, and dyslipidemia with plasma active ghrelin levels in patients with T2DM.[23]

Regarding blood pressure, lipid profile, and FBS, all showed no significant differences between the genotypes even when genotypes were combined. In this study, no differences in the plasma levels of leptin between the genotypes were observed. Moreover, plasma levels of ghrelin were lower in A carriers compared with C/C mutant homozygotes also; AA and AC genotypes (A carriers) were associated with low ghrelin levels.[12] In the previous study by Vartiainen et al., fasting plasma total ghrelin concentrations were not associated with the SNP -501A/C genotypes, proposing that this SNP is not playing a major role in the global determination of fasting ghrelin plasma levels. It must be reminisced that the plasma concentration of any peptide hormone is not a perfect consideration of the rate of its gene expression for the reason that events such as storage and release of the hormone participate in the determination of the plasma hormone levels.[12] However, there were no significant changes in the important characteristics associated with T2DM such as lipid and glucose metabolism in these two groups of samples. Therefore, it is hard to conclude that the ghrelin gene's SNP 501A/C is associated with Saudi patients with T2DM. The authors found that expression of ghrelin and some characteristics such as TG, T-CHOL, and other parameters associated with T2DM have been changed in the cases group compared with the control group but could not exclude the possibility that these changes may be attributed by other causes or other SNP instead of single SNP 501A/C. Therefore, major concerns will be focused on the study of other SNPs besides single 501A/C polymorphism in ghrelin in the selected population and to study their possible association with T2DM.

Limitation of Study

Our study is limited by relatively low numbers of participants; therefore, our results of genetic analyses need to be interpreted with caution and should be replicated in future studies with much larger sample numbers. Limitations of this study also include the limited amount of data available about the genetic contribution of ghrelin on metabolic syndrome among Arabs; thus, we were unable to compare our results with individuals from same ethnicity.

Conclusions

Ghrelin gene -501A/C polymorphism has no significant relationship with the susceptibility of T2DM. However, plasma ghrelin was negatively correlated with blood pressure, FBS, TG, and T-CHOL in the patients with diabetes. On the other hand, plasma ghrelin showed a significant positive correlation with HDL cholesterol. Plasma levels of ghrelin were lower in A carriers compared with C/C mutant homozygotes and A carriers were associated with lower ghrelin levels.

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References

1. International Diabetes Federation. IDF Diabetes Atlas. 6th edn. Brussels, Belgium: International Diabetes Federation, 2013.
2. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999;402:656–60.
3. Ukkola O. Ghrelin and metabolic disorders. Curr Protein Pept Sci. 2009;10:2–7.
4. Pöykkö SM, Kellokoski E, Hörkkö S, Kauma H, Kesäniemi YA, Ukkola O. Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes. 2003;52:2546–53.
5. Barazzoni R, Zanetti M, Ferreira C, Vinci P, Pirulli A, Mucci M, et al. J Clin Endocrinol Metab. 2007;92:3935–40.
6. Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, et al. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab. 2002;87: 240–4.

7. Østergård T, Hansen TK, Nyholm B, Gravholt CH, Djurhuus CB, Hosoda H, et al. Circulating ghrelin concentrations are reduced in healthy offspring of type 2 diabetic subjects, and are increased in women independent of a family history of type 2 diabetes. Diabetologia. 2003;46:134–6.
8. Mager U, Lindi V, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, et al. Association of the Leu72Met polymorphism of the ghrelin gene with the risk of type 2 diabetes in subjects with impaired glucose tolerance in the Finnish Diabetes Prevention Study. Diabet Med. 2006;23:685–9.
9. Perret J, De Vriese C, Delporte C. Polymorphisms for ghrelin with consequences on satiety and metabolic alterations. Curr Opin Clin Nutr Metab Care. 2014;17(4):306–11.
10. Benso A, Casanueva FF, Ghigo E, Granata A Eds. The Ghrelin System. Endocr Dev. Vol. 25Basel: Karger, 2013. pp. 25–40.
11. Jörg K, Sonja D, Alfried K, Thomas V. Metabolic and health implications of moderate ketosis and the ketogenic diet-effects of the ketogenic diet in the glucose transporter 1 deficiency syndrome. Prostaglandins Leukot Essent Fatty Acids. 2004;70:321–7.
12. Vartiainen J, Kesaniemi YA, Ukkola O. Sequencing analysis of ghrelin gene 5-flanking region: relations between the sequence variants, fasting plasma total ghrelin concentrations, and body mass index. Metabolism. 2006;55:1420–5.
13. Ali TM, Alhazmi AS, Khalifa AS. Association of single nucleotide polymorphism of 5-flanke ghrelin gene with obesity in Saudi subjects. IOSR J Dent Med Sci. 2014;13:30–5.
14. Carroll JJ, Smith N, Babson AL. A colorimetric serum glucose determination using hexokinase and glucose-6-phosphate dehydrogenase. Biochem Med. 1970;4:171–80.
15. Dietschy JM, Weeks LE, Delente JJ. Enzymatic assessment of free and esterified cholesterol levels using the oxygen electrode in a modified glucose analyzer. Clin Chem Acta. 1976;73:407–14.
16. McGowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin Chem. 1983;29:538–42.
17. Assman G, Schriewer H, Schmitz G, Hagele EO. Quantification of high density lipoprotein cholesterol by precipitation with phosphotungestic acid/MgC12. Clin Chem. 1983;29:2026–30.
18. Friedwald WT, Levy RI, Fridrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifugation. Clin Chem. 1972;18:449–502.
19. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2003;26: S5–S20.
20. Jang YS, Hwang DJ, Yang YJ, Park JH, Lee DY. Serum ghrelin concentrations in type 2 diabetes mellitus. J Korean Soc Pediatr Endocrinol. 2004;9:59–65.
21. Tschop M, Weyer C, Tataranni PA, Devanarayan V, RavussinE, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes. 2001;50:707–9.
22. Katsuki A, Urakawa H, Gabazza EC, Murashima S, Nakatani K, Togashi K, et al. Circulating levels of active ghrelin are associated with abdominal adiposity, hyperinsulinemia and insulin resistance in patients with type 2 diabetes mellitus. Eur J Endocrinol. 2004;151:573–7.
23. Poykko SM, Kellokoski E, Horkko S, Kauma H, Kesaniemi YA, et al. Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes. 2003; 52:2546–53.

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