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Association of *ADIPOQ* +45 T/G Polymorphism with Circulating Adiponectin Levels and Obesity in Iraqi Women with Gestational Diabetes Mellitus

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Abstract

The dysregulation of pro- and anti-inflammatory adipokines contributes to insulin resistance which is the main feature of the pathogenesis of gestational diabetes mellitus (GDM). This study aimed to examine the potential association of circulating concentrations of adiponectin- anti-inflammatory adipokine- and the *ADIPOQ* (rs2241766 T/G) polymorphism with obesity and the risk of developing GDM in the Iraqi population. 50 pregnant women diagnosed with GDM and 50 healthy pregnant women who were attending the Pregnancy Care and Maternal Medicine Outpatient Departments at Baghdad Teaching Hospital, Medical city, Baghdad/Iraq were enrolled in this study from December 2022 to May 2023. Serum adiponectin levels were estimated by immunological assay. Genotyping of *ADIPOQ* (rs2241766) polymorphism was performed by Taqman Real Time polymerase chain reaction (RT-PCR) technique. The (TG+GG) genotype at the rs2241766 conferred an increased risk of GDM with 41 (82%) of GDM women had TG+GG compared to 20 (40%) of normal pregnant women: [odds ratio (OR): 6.8; 95% confidence interval (CI) 2.7317 to 17.0935; *P*<0.001]. Compared with women in the highest quartile of total adiponectin, women in the median (50th) quartile had a fourfold increase in risk of GDM: [Odd ratio (OR): 4.093; 95% CI: (1.160 to 14.433); P<0.05]. Women who had the G allele at the *ADIPOQ* rs2241766T/G may be more predisposed to develop GDM due to hypoadiponectinemia, irrespective of maternal Body Mass Index (BMI). Low adiponectin levels were associated with a BMI of ≥30 kg/m^2, suggesting an influential role of the anti-inflammatory adipokine on GDM risk and insulin sensitivity.

Keywords: Adipokines, Glucose intolerance, Hyperlipidemia, Hypoadiponectinemia,Obesity,Pregnancy.

Introduction

Gestational diabetes mellitus (GDM) is a condition of glucose metabolism disorder that develops during pregnancy. It is characterized by varying degrees of hyperglycemia in women without a history of diabetes. This condition typically occurs in the second or third trimester of pregnancy [1]. Globally, the prevalence of GDM exhibits a significant variation, with an estimated global prevalence of 14.0% using the International Association of Diabetes and Pregnancy Study Groups (IADPSG) diagnostic protocol [2], which involves a 2-hour, 75-gram oral glucose tolerance test (2-h 75-g OGTT) where GDM is diagnosed if specific glucose thresholds are surpassed: fasting plasma glucose levels > 92 mg/dL, 180 mg/dL at one hour, and 153 mg/dL at two hours [3]. While there is no current official statistical data on GDM incidence in Iraq, a previous study conducted by Mohammed in 2020 estimated a GDM prevalence of 13.3% based on glycated hemoglobin (HbA1c) values ranging from 5.7% to 6.4% [4]. Although OGTT is recommended as the standard diagnostic test for GDM by international

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organizations, in Iraq, HbA1c of ≥7.5% is frequently employed for the GDM diagnosis. Bozkurt *et al.*, identified impaired glucose metabolism in pregnancy at 5.7% or above (about 37 mmol/mol) [5].
GDM remains a critical health concern, particularly among pregnant women with obesity. The pathophysiology of GDM involves a combination of hormonal, genetic, and environmental factors, but impaired insulin sensitivity coupled with β-cell dysfunction and insufficient glucose sensing to stimulate insulin secretion and maintain normoglycemia are the primary features of the pathogenesis of GDM [6]. The pathogenesis of GDM involves a complex interplay of factors, including a dysregulation of adipokines a group of inflammatory cytokines and hormones released by the adipose tissue, which can contribute to insulin resistance, where pro-inflammatory adipokines can impair insulin secretion from β-cells by inhibiting insulin signaling pathway, resulting in insulin resistance [7]. GDM is characterized by elevated circulation levels of pro-inflammatory cytokines such as TNFα, IL-6, IL-8, and IL-1β [8,9] and leptin, with a corresponding decline in concentrations of adiponectin [10]. Among these adipokines, adiponectin plays a crucial role in counteracting insulin resistance. Adiponectin enhances insulin sensitivity in the peripheral cells. The insulin-sensitizing effects of adiponectin are attributed to its anti-inflammatory properties. Adiponectin reduces inflammation and regulates glucose and lipid metabolism by activating two key pathways: AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptors (PPARs) via binding to seven transmembrane receptors known as AdipoR1 and AdipoR2 leading to higher fatty acid oxidation and decreased obesity [11]. Accordingly, a dysregulation of adiponectin is linked to metabolic disorders such as insulin resistance and obesity. Many studies have linked hypoadiponectinemia, a decrease in adiponectin levels, to β-cell dysfunction, especially during pregnancy, which is of particular relevance in the context of the pathophysiology of GDM [12].
Adiponectin is encoded by the *ADIPOQ* gene, which is referred to as adipose most abundant gene transcript 1 (*apM1*) and is exclusively expressed in the adipose tissue. *ADIPOQ* gene is located on chromosome 3q27 region; this region is associated with various genetic factors related to obesity and metabolic disorders. The *ADIPOQ* gene is composed of three exons and two introns, spanning a total of approximately 16 kilobases (kb) of genomic DNA sequence [13]. The single nucleotide polymorphism (SNP) rs2241766 +45 T/G is located in exon 2 of the *ADIPOQ* gene and causes a T to G substitution at position 45 [14]. The polymorphism of this location influences insulin sensitivity because of its potential polymorphic effects on the stability or shearing of precursor mRNA and adiponectin concentration [15]. The +45T/G (rs2241766) variant in the *ADIPOQ* gene has been previously found to be associated with alterations in the level and activity of adiponectin and so can significantly influence the pathogenesis of diabetes including GDM [16]. In this study, we delve into the association between the *ADIPOQ* +45T/G variant and hypoadiponectinemia and GDM susceptibility among obese Iraqi women.

Materials and methods

Experimental design
This is a case-control study. The study was conducted between November 2022 and May 2023. 50 pregnant women diagnosed with GDM, acted as (patient group) and 50 healthy pregnant women served as (control group) were randomly selected while attending their routine antenatal checkups at the Outpatient Clinic of Maternity Medicine and Pregnancy Care at Baghdad Teaching Hospital. The pregnant subjects aged (17-43) years and were in their (14-40) weeks of gestation. The diagnosis of GDM in this study was determined by obstetricians based on two criteria: HbA1c Levels: Pregnant women with HbA1c levels exceeding 5.7% were identified as having impaired glucose metabolism during pregnancy and a potential risk for GDM and Ultrasonic Marker of Excessive Amniotic Fluid which refers to an excessive amniotic fluid (polyhydramnios). Polyhydramnios, detected through ultrasound examination, is an abnormal increase in the amniotic fluid volume surrounding the fetus.

Ethical Approval
The Ethics Committee at University of Baghdad reviewed and approved the study protocol under reference number (3176 ⁄ 2022) and in accordance with the regulations of Medical City Directorate, the Ministry of Health in Iraq granted ethical approval for this study with reference number 52340-12/12/2022.
Type of sampling and reasons for selection

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Simple random sampling was used for obtaining a representative sample where every member of the study population has an equal chance of being selected. This helps in obtaining a representative sample and generalizing findings to the entire population in Iraq.
A combination of both quantitative and qualitative methods was used in this study with the aim to provide a more comprehensive understanding of the pathogenesis of GDM.

Patients' consent

After receiving approval from the Gynecological Outpatient Clinic of Baghdad Teaching Hospital, a questionnaire was used to collect the participants' data regarding their current health status and medical history. All participants gave their verbal consent to participate in this study after they were provided with a thorough explanation of the research protocols and objectives, ensuring that they were informed about the purpose and procedures of the study. This practice aligns with ethical standards to obtain informed consent and respect the autonomy of the individuals involved in the research. Participants were informed that their data would not be disclosed outside the research, emphasizing the confidentiality and privacy of their information as part of ethical considerations.

Inclusion and exclusion criteria

Inclusion criteria

For the study, GDM women were sought out irrespective of their reproductive age, gestational age or pregnancy BMI. The control group consisted of apparently healthy pregnant women without previous gestational or chronic diabetes.

Exclusion criteria

Women presented with pre-existing diabetes confirmed with HbA1c greater than 6.5% (48 mmol/mol) and fasting glucose of ≥ 7.0 mmol/L, other chronic diseases or pregnancy-related complications were excluded. The controls were subjected to the same exclusion criteria as the GDM subjects.

Collection and storage of samples

Each subject's blood was drawn via venipuncture by placing a tourniquet around the arm just above the elbow. After a 12-h fast, 5 ml of venous blood were drawn from each participant. For the measurement of all biochemical parameters, 3 ml of venous blood were dispensed into a serum separator gel tube. Serums were separated by centrifugation at 3000 rpm for 5 minutes. 2 ml of extra venous blood were dispensed into an EDTA tube for DNA extraction. Serum and whole blood samples were stored under (-20 °C) until being analyzed.

Analytical methods

Standard enzymatic methods were used to measure fasting Total cholesterol (CHOL), triglyceride (TRIG), and high-density lipoprotein (HDL) were measured on 3 ml of the fresh samples transferred to Gel separator tubes centrifuged at 3000 rpm to obtain serum, using Abbott ARCHITECT c4000 Clinical Analyzer and reagents. Serum adiponectin level was measured by enzyme linked immunosorbent assay (ELISA) method using Human adiponectin ELISA kit provided by provided by BT LAB, China.
Genomic DNA was extracted from the remaining 3 ml EDTA-treated whole blood using AddPrep Genomic DNA Extraction Kit (AddBioMediTek Co., Korea). The purity and concentration of the DNA templates were then measured with Nanodrop spectrophotometer (Thermo Fisher Scientific, USA). The purity ranged from 1.8 to 1.9 and the DNA concentration from (60-110) ng/ µl. Gel electrophoresis was used to assess the integrity of DNA templates for subsequent accurate determination of genotypes and allele frequencies of the target 45T/G rs2241766 polymorphism in *ADIPOQ* gene in GDM and non-GDM control subjects with TaqMan real-time PCR as shown in Fig. 1.

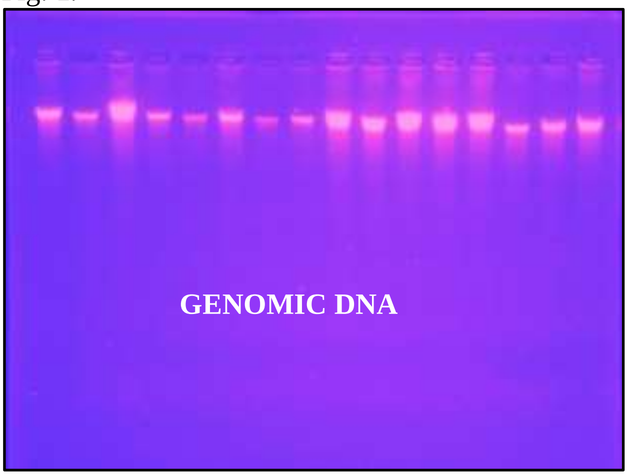

Figure 1. DNA Electrophoresis under UV light; the extracted genomic DNA samples were loaded in 1% agarose gel for 90 min at 70 volts. DNA bands were visualized under UV light. Intense color indicates (high quality DNA).

The target rs2241766 SNP in *ADIPOQ* gene was

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amplified by TaqMan probe-based real-time PCR (Qiagen, USA), using *ADIPOQ* +45TG forward primer: (5'-GCCATCCAACCTGTGCAG-3') and wild allele-specific probe and *ADIPOQ* +45TG reverse primer: (5'-GTCTCTCCATGGCTGACAGT-3'), and mutant allele specific probe with a 5'-end marked with (VIC) and 3'-end with BHQ. A 20µl reaction master mix was made up of 10µl of PerfectStart II Probe qPCR SuperMix UDG (TransGen Biotech, China), 2 µl of both forward and reverse primers (10 pmole/µl working solution), 2 µl of both wild and mutant probes (10 pmole/µl working solution), 3 µl of DNA template, with the remaining volume being nuclease-free water NFW. The PCR protocol applied included initial 5 cycles with a pre- denaturation step of 95°C for 1 min, denaturation at 95°C for 10 s, annealing at 58 °C for 20 s, and extension acquiring on Green and Yellow (FAM and VIC) channels at 72 °C for 30 s. followed by 35 cycles. The generation of green fluorescence, the Green Channel "FAM" with no markers, during amplification signifies homozygous wild types, and the yellow fluorescence, the yellow channel "VIC" with circles, indicates homozygous mutants. In cases where both green and yellow fluorescence are observed, it suggests the presence of heterozygotes as illustrated in Fig. 2.

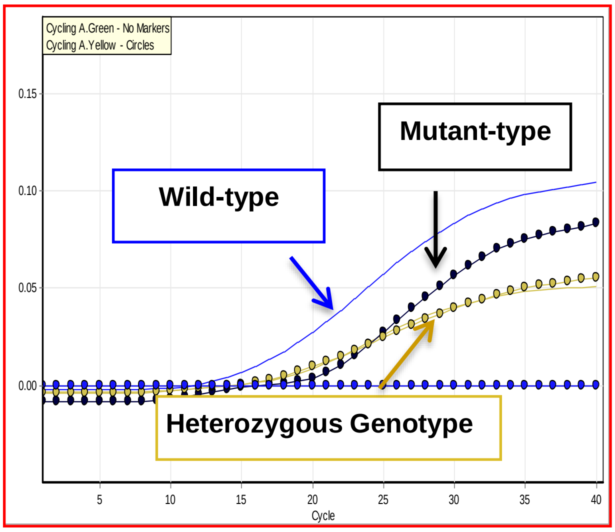

Figure 2. TaqMan probe-based SNP genotyping results show Wild (Blue), mutant (Black) and heterozygous (Brownish Yellow)

Statistical Analysis

Statistical data was analyzed using IBM SPSS Statistics (version 26). Continuous variables were expressed as mean ± standard error (±S.E.). A two-side probability (*P*) value set at ≤ 0.05 and ≤0.01 was considered significant. Student's t-test and One-way analysis of variance (ANOVA) were performed to compare between means. Chi Square $\chi 2$ test was used for analyzing categorical variables. Multinomial Logistic regression models were used to obtain odds ratios (ORs) with 95% confidence interval (CI) and to estimate the relative risk of GDM.

Results

Maternal Characteristics

Baseline demographic and clinical characteristics for the women with GDM (n=50) and for normal pregnant women (n=50) are described in (Table 1). The GDM group, had a significantly higher mean age of 30.56±0.87 years than 26.50 ±0.86 years for the non-GDM healthy pregnant women; (*P*= 0.001**) with the majority 40 (80.0%) of GDM women aged above 25 years; [OR: 4.7; 95% CI: (1.931-11.418); *P*≤0.01**]. The mean BMI ±S.E. was 32.38± 0.87 kg/m^2 for GDM women and 28.98± 0.65 kg/m^2 for non-GDM control subjects and there was a significant association between GDM and BMI in pregnancy (*P*=0.009**). GDM rate was significantly higher among pregnant women 47 (94.0%) who had a pregnancy BMI of ≥ 25 kg/m^2 compared to 11 (22.0%) women who had no GDM while only 3 (6.0%) of women with normal BMI <25 kg/m^2 developed GDM. Results from the present study showed that overweight and obese women defined by having a BMI ≥25 kg/m^2 were at fourfold higher risk for developing GDM compared to normal-weight women [OR: 4.419; 95% CI:(1.151-16.966); *P*=0.02**]. Additionally, there was 28.2 times higher risk of hypertension among women with GDM [OR: 30.032; 95% CI (3.826 - 235.767); *P* <0.001**]. The prevalence of GDM in this study was observed higher in the third trimester of pregnancy where most of GDM women 37 (74.0%) were between months (7-9) of pregnancy. Yet, no statistically significant difference across both groups was detected; *P*> 0.05

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Table 1. Clinical Characteristics of GDM and Non-GDM healthy pregnant subjects

Variables	GDM (N =50)	Non-GDM (N = 50)	OR (95% CI)	*P*
Age (year)				
≤25	10 (20.0%)	27 (54.0%)		
>25	40 (80.0%)	23 (46.0%)	**4.7 (1.931 to11.418)**	**0.001****
Mean± S.E.	30.56±0.87	26.50±0.86		
BMI (kg/m2)				
<25	3 (6.0%)	11 (22.0%)		
≥ 25	47 (94.0%)	39 (78.0%)	**4.419 (1.151 - 16.966)**	**0.009****
Mean± S.E.	32.38± 0.87	28.98± 0.65		
Gestational Hypertension				
Yes	19 (38.0%)	1 (2.0%)		
No	31 (62.0%)	49 (98.0%)	**30.032 (3.826-235.767)**	**<0.001****
Gestational Age				
1st trimester	4 (8.0%)	5 (10.0%)	----	
2nd trimester	9 (18.0%)	8 (16.0%)	-----	
3rd trimester	37 (74.0%)	37 (74.0%)	-----	
mean± S.E.	7.20 ±0.304	7.42±0.313		**>0.05 NS**

Notes: * *P* value<0.05 indicates statistical significance. Age subgroups include ≤25 and > 25 years. Gestational age was classified into the three following subgroups or **three-month periods (trimesters): 1st trimester: 6 weeks to 13 weeks + 6 days, 2nd trimester: 14 weeks to 27 weeks + 6 days, and 3rd trimester: 28 weeks to 41 weeks + 6 days**. BMI was further divided in the two following subgroups: <25 and ≥25 kg/m^2.

Clinical Implications of Adiponectin and Lipid Levels in GDM

The mean concentrations of fasting lipid levels displayed no significant difference across the study groups in accordance with Hossain et al.,[17]. Yet GDM women had considerably higher mean TRIG ±S.E. levels 209.580±11.47 compared to non-GDM healthy women 179.85±11.479 mg/dL, yet this difference did not reach a statistical significant difference (*P* >0.05). Moreover, plasma concentration of adiponectin was the same across the GDM and non-GDM groups (15.3576±0.361 mg/L versus 15.933 ±0.621 mg/L; *P* >0.05). Results of lipid and adiponectin levels are depicted in Table 2.

Table 2. Differences in maternal lipid profile and circulating adiponectin levels between study groups

Parameters	GDM subjects	Non-GDM Control subjects	*P*
	Mean ± S.E.	Mean ± S.E.	
CHOL(mg/dl)	204.136± 6.92	204.720±7.2226	0.954NS
TRIG(mg/dl)	209.580±11.47	179.85±11.479	0.070 NS
HDL(mg/dl)	48.93± 1.74	51.840±1.411	0.196 NS
Adiponectin (mg/L)	15.3576±0.361	15.933 ±0.621	0.426 NS

NS indicates Non-Significant; *P*>0.05

Correlation of Hypoadiponectinemia with Maternal Obesity

Table 3. Risk estimate (ORs 95% CI) for GDM based on quartiles of adiponectin levels

ADIPOQ Quartiles mg/L	GDM	Non-GDM	Risk for developing GDM	
	N	**N**	**OR (95%CI)**	***P*-value**
1st Quartile <14.070	14	8	2.528 (0.750-8.522)	0.14
2nd Quartile (14.070-15.670)	10	13	1.111 (0.340-3.631)	0.86
3rd Quartile (>15.670-17.360)	17	6	4.093 (1.160-14.433	0.03*
4th Quartile >17.360	9	13	1.00 (Reference)	----

Reference =**Non-GDM Group** ; * *P*-value ≤ 0.05 refers to "**statistically significant**"

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The pregnant women were further categorized into four quartiles in order to assess the diagnostic power of adiponectin in predicting GDM risk across different ranges. The quartile categories were labeled as the lowest quartile (1st Quartile or Q25th) which represents the lowest 25th percentile and comprised women with adiponectin levels below 14.0700 mg/L, the middle quartile (2nd Quartile or Q50th) which, represents 50th percentile where half of the pregnant subjects have adiponectin levels within the range of (14.070 - 15.670) and the third quartile (3rd Quartile), which represents the circulating values set at (>15.670-17.360) mg/L while the upper quartile or (4th Quartile) which represents the upper 25th percentile and comprised individuals with adiponectin levels greater than 17.3600 while three-quarters of pregnant subjects had adiponectin levels below this threshold. Multinomial logistic regression analysis was carried out to assess associations between the risk to developing GDM and adiponectin ranges.

Table 3 shows that the likelihood of GDM risk was 2.528-fold more higher among pregnant women in the lowest Q25th who had adiponectin levels of <14.0700 mg/ L [OR: 2.528; 95% CI: (0.750-8.522); $P > 0.05$*] whereas women in the Q50th with adiponectin levels ranging between (14.070-15.670) mg/L had lower risk for GDM: [OR: 1.111; 95% CI: (0.340-3.631); $P > 0.05$]. In contrast, women who developed adiponectin levels less than 17.3600 mg/L were at a four more times higher risk of GDM: [OR: 4.093; 95% CI: (1.160-14.433); $P = 0.028$*].

In the present study, we also explored the association between adiponectin, BMI and lipid profile during pregnancy. After adjustment for adiponectin quartiles, results showed that GDM women in the 3rd quartile of adiponectin had a BMI of >30 kg/m2 and developed comparably higher triglyceride levels 223.720±22.66 compared to healthy control women in the matched quartile who had a BMI <30 kg/m2 and lower triglycerides levels of 146.667±24.316 mg/dL as shown in Table 4.

Table 4. Comparison between BMI and serum lipid levels across the study groups based on quartiles of adiponectin

	Statistics	Adiponectin (mg/L)				
Groups		**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**	***P***
GDM	BMI	30.792±1.599	30.630±1.879	34.277±1.675	33.199±1.588	0.327
	CHOL	225.071±12.7234	176.078±14.249	200.114±10.535	207.222±18.601	0.120
	TRIG	212.643±17.426	165.792±27.779	223.720±22.66	221.889±22.561	0.337
	HDL	50.143±4.236	45.794±2.279	50.612±2.233	47.0±5.164	0.744
Non-GDM	BMI	29.310±2.269	29.2277±0.972	29.7183±1.620	28.8723±1.579	0.989
	CHOL	206.375±14.813	212.0±14.256	196.167±15.972	185.077±12.919	0.502
	TRIG	211.50±38.695	187.846±26.02	146.667±24.316	165.823±19.358	0.495
	HDL	52.50±3.229	55.3846±2.714	52.0±3.276	50.385±3.661	0.697

Association of the *ADIPOQ* +45T/G (rs2241766) SNP with GDM

The +45T/G (rs2241766) polymorphism in *ADIPOQ* gene was significantly associated with a higher GDM risk in the heterozygous (TG) and homozygous mutant (GG) genotypes compared to TT genotype. Out of the 50 pregnant women with GDM, 35 (70%) carry the TG genotype compared to the 20 (50%) of the 50 healthy pregnant women with the same genotype: [OR: 5.8; 95% CI: (2.3117-14.7197); $P < 0.001$**]. The GG genotype of +45T/G SNP was less frequent with only 6/50 (12%) GDM women carry GG genotype whereas the GG genotype was absent in the non-GDM group: [OR: 41; 95% CI: 2.1471 to 81.2937; $P = 0.01$*]. 41 (82%) out of 50 GDM women compared to 20 (40%) of the 50 healthy control women exhibited the dominant model "TG+GG": [OR: 6.8; 95% CI:(2.7317-17.0935); $P \leq 0.001$**]. Comparing the distribution of allele T and allele G in +45T/G SNP among the GDM and non-GDM women, data from this study showed that G allele in +45T/G SNP was significantly more frequent (47%) in GDM women than (20%) in healthy pregnant women: [OR: 3.5; 95% CI: (1.8931-6.6466); $P \leq 0.001$**]. The study population showed no statistical significant in the recessive "TT + TG" model; $P > 0.05$.The genotype distributions of SNP are summarized in Table 5.

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Table 5. Distribution of study subjects with and GDM based on genotypes and alleles at the *ADIPOQ* +45T / G gene polymorphism

ADIPOQ +45T /G Genotype	Frequencies (%) GDM (n= 50)	Non-GDM (n= 50)	P	Odd ratio (95% CI)
		Co-dominant		
TT	9 (18%)	30 (60%)	---	1.00 (Reference)
TG	35 (70%)	20 (50%)	**0.0002****	5.8 (2.3117 to 14.7197)
GG	6 (12%)	0	**0.01***	41 (2.1471 to 81.2937)
		Dominant		
TT	9 (18%)	30 (60%)	---	1.00 (Reference)
TG+ GG	41 (82%)	20 (40%)	**0.0001****	6.8 (2.7317 to 17.0935)
		Recessive		
TT +TG	44 (88%)	50 (100%)	---	1.00 (Reference)
GG	6 (12%)	0	0.06	14.7 (0.8080 to 26.3519)
		T / G Allele		
T	0.53 (53%)	0.8 (80%)	---	1.00 (Reference)
G	0.47 (47%)	0.2 (20%)	**0.0001****	3.5 (1.8931 to 6.6466)

Impact of *ADIPOQ* +45T / G SNP on adiponectin concentrations in GDM

The present study showed that GDM women who carried the TG and GG genotypes of *ADIPOQ* +45T/G SNP developed considerably lower mean adiponectin levels of 15.2± 0.453 and 15.37± 0.80, respectively, than 16.00 mg/L in GDM Yet, the rs2241766 genetic variant does not appear to significantly impact adiponectin levels among both GDM and non-GDM subjects. The distribution of adiponectin levels across the genotypes of *ADIPOQ* +45T/G SNP is depicted in Table 6.

Table 6. Correlation between serum adiponectin levels and rs2241766 T/G SNP genotypes across the GDM and non-GDM groups

Groups	*ADIPOQ* +45T / G Genotype	Total Adiponectin mg/L Mean ± S.E.	P
GDM	**TT**	15.9± 0.86	0.78
	TG	15.217± 0.453	
	GG	15.367± 0.80	
		Dominant Model	
	TG+ GG	15.24± 0.400	0.48
Non-GDM	**TT**	16.14± 0.73	0.69
	TG	15.63± 1.1	
		Dominant Model	
	TG+ GG	15.63±1.13	0.7

Discussion

Gestational diabetes mellitus (GDM) and adiposity (obesity) are interconnected health conditions that can influence each other. Obesity in pregnancy can lead to insulin resistance, disrupt glucose metabolism, causing further dyslipidemia, and inflammation and so increasing the risk of GDM [18,19]. This study observed an increased likelihood of developing GDM in women aged over 25 years, in line with findings from another study by Meng et al.,[20]. The rationale provided is that there is a natural increase in insulin resistance as women age, especially beyond the age of 25. In accordance with previous studies, findings from the present study demonstrated that a high BMI is an independent risk factor for GDM [21]. Similar to Read et al.,[22], the risk of GDM increased progressively with advancing gestational age from 8.0% in 1st trimester, and 18.0% in 2nd trimester, up to 74.0% in the third trimester of pregnancy, emphasizing the universal screening at 24-28 weeks recommended by IADPSG and WHO. This timing aligns with the physiological changes supporting fetal growth, often leading to increased insulin resistance in pregnant women. However, inadequate compensation by pancreatic β-cells can result in elevated blood glucose levels, posing risks for GDM development.

Recent studies consistently report that women with GDM face a significantly increased risk of

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hypertension [23]. Maternal hypertensive disorders during pregnancy and GDM share common pathogenic pathways, including insulin resistance, endothelial dysfunction and an increase in pro-inflammatory markers. Both conditions are associated with inflammatory response [24].

Interestingly, our findings contradict previous studies associating GDM with hyperlipidemia [25]. The lack of a significant difference in lipid levels between women with and without GDM emphasizes that lipid metabolism may not be as directly impacted by GDM in the studied population.

This study supports the hypothesis that has previously been proposed that the reduction of maternal adiponectin levels is linked to insulin resistance among Iraqi women with GDM [26]. Insulin resistance in GDM is worsened by a dysregulation in adipocyte-derived hormones and cytokines, specifically referred to as adipokines. Adiponectin is an anti-inflammatory adipokine that plays a crucial role in regulating glucose and lipid metabolism. In pregnant women, circulating adiponectin levels decrease correspondingly with insulin sensitivity [27]. This study found no statistically significant difference in means of adiponectin levels between GDM and non-GDM healthy women under t-test, (P >0.05). Similar findings were observed by Tangjittipokin et al.,[28]. After categorizing the women into quartiles of adiponectin values, this study showed that women in the 3rd quartile with adiponectin concentrations between (>15.670-≤17.360) mg/L were four times more likely to develop GDM compared with women in the highest quartile of total adiponectin, In agreement with Hedderson et al., [29], the risk of GDM appears to increase with decreasing quartile. Balachandiran et al., revealed consistent results [30]. Plasma adiponectin concentration in humans ranges between 5 and 30 µg/mL [31]. The results described in this study showed that women with decreased adiponectin levels were predisposed to develop GDM. Such findings support the hypothesis that low levels of adiponectin were associated with an increased risk of GDM as reported in many previous studies [32,33]. Adiponectin levels normally decrease during pregnancy associated a progressive decrease in insulin sensitivity to enhance physiologic insulin resistance. However, women with a pre-gestational insulin resistance develop further lower adiponectin levels accompanied with decreased glucose uptake, pancreatic β- cell dysfunction, and hyperglycemia and subsequently develop GDM [34]. Results from the present study suggest that lower levels of adiponectin are correlated with a higher likelihood of developing GDM independent of BMI. Many other studies found a strong correlation between low adiponectin levels and a high BMI [35]. In the present study, women with adiponectin levels falling within the 3rd and 4th quartile had values ranging from >15.670 to 17.360 mg/L and greater than 17.360 mg/L, respectively, had higher BMI and higher triglycerides levels compared to normal pregnant women. These findings suggest that higher levels of adiponectin are associated with increased BMI and provide insights into a potential interplay between BMI, adiponectin, and lipid levels in GDM.

The analysis of ADIPOQ +45T/G rs2241766 genotypes showed that women carrying "GG" and 'TG" genotypes were at higher risk for developing GDM. Similarly, a recent study, conducted on Saudi women, demonstrated that the dominant model "TG + GG", and heterozygous (TG) and homozygous (GG) genotypes of the rs2241766 +45T/G SNP conferred an increased risk of GDM. Similar to the findings in this study, Alshammary et al., also observed no significant link was observed between the rs2241766 +45T/G polymorphism the recessive genetic model "TT + TG" [36]. The lack of statistical significance in the recessive model ("TT + TG") suggests that 'T' allele does not confer a risk for GDM and the "G" allele. This could also indicate a potential link between allele "G", defined as the “risk allele”, at ADIPOQ +45T/G variant and hypoadiponectinemia in GDM. Another study also identified a correlation between the presence of G allele and TG and GG genotypes at the ADIPOQ +45T/G SNP with GDM risk in Chinese population [37]. Contradicting findings were reported by Nezamzadeh et al., who found that Iranian women carrying T allele and TT genotype had a higher likelihood of developing GDM [38].

There have been limited studies that simultaneously investigated the association between the rs2241766 SNP and serum adiponectin levels in pregnancy complicated by GDM. In accordance with Fernandez et al., [39], this study showed that ADIPOQ +45T/G rs2241766 SNP did not influence plasma adiponectin levels among both GDM and non-GDM subjects. The lack of impact suggests that BMI and other environmental factors play a more dominant role in determining adiponectin levels in the study population. Yet women in this study with "GG" and "TG" genotypes developed considerably lower adiponectin levels, in agreement with Huang et al., who suggested a potential association between the TT genotype and elevated adiponectin levels [40].

Conclusion

In conclusion, the present study found that pregnant women carrying the G allele at the +45T/G rs2241766 gene variant had a higher risk of developing GDM, and lower adiponectin levels were associated with increased GDM risk, especially when concentrations fell below 17.3600 mg/L. These results imply a potential interplay of genetic and biochemical factors in the development of GDM. The findings of this study underscore the importance of further investigations on the factors influencing GDM to address potential limitations. Larger sample sizes and longitudinal study designs are recommended for improved GDM prevention and management strategies.

Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee at the University of Baghdad.
- No animal studies are present in the manuscript.
- No potentially identified images or data are present in the manuscript.

Authors' contribution statement

R.A. confirms sole responsibility for the following: data collection, analysis and interpretation of results and manuscript preparation. D. H. supervised and validated the current research outputs. All authors contributed to the study conception and design. All authors reviewed the results and approved the final version of the manuscript.

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العلاقة بين التغاير الجيني *ADIPOQ* +45 T/G ومستويات الأديبونيكتين مع السُمنة لدى النساء العراقيات المصابات بداء السكري الحملي

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الخلاصة

يساهم الخلل في مستويات الأديبوكينات المحفزة والمضادة للإلتهابات في مقاومة الأنسولين والتي تعد السمة الرئيسية للتسبب في داء السكري الحملي. تهدف هذه الدراسة إلى الكشف عن العلاقة بين تركيز الأديبوكاين المضاد اللإلتهابات (الأديبونيكتين) في الدم و التغاير الجيني للمورثة (*ADIPOQ* rs2241766 T/G) مع السُمنة وخطر الإصابة بداء السكري الحملي لدى النساء العراقيات. شملت هذه الدراسة 50 امرأة حامل تم تشخيص إصابتهن بداء السكري أثناء الحمل و 50 امرأة حامل سليمة ممن كانوا يراجعون إستشارية رعاية الحوامل وطب الأمومة في مستشفى بغداد التعليمي، مدينة الطب، بغداد / العراق للفترة بين ديسمبر 2022 و مايو 2023. تم تقديرمستويات الأديبونيكتين في الدم بالمقايسة الامتصاصيَّة المناعيَّة للإنزيم المرتبط و تم استخدام تقنية تفاعل البلمرة المتسلسل بالزمن الحقيقي RT-PCR في التنميط الجيني للمورثة rs2241766T/G. و كانت النتائج كالآتي: إرتبط النمط الجيني (TG+GG) للمورثة rs2241766 T/G بزيادة خطر الإصابة بالسكري الحملي حيث 41 (82%) من مجموع 50 مريضة بالسكري الحملي كان لديهم التعبير الجيني (TG+GG) مقارنة بـ 20 (40 %) من الحوامل السليمات و كان معامل الخطر النسبي = (6.8)؛ و نسبة الأرجحية 95% CI تتراوح بين 2.7317 و 17.0935؛ عند مستوى معنوية < 0.001**]. و كانت النساء الحوامل التي تتراوح تراكيز الأديبونكتين لديهن بين (<15.670-17.360) أكثر عرضةً للإصابة بالسكري الحملي بمقدارأربعة أضعاف; حيث كان معامل الخطر النسبي= 4.093؛ و نسبة الأرجحية 95% CI تتراوح بين (14.433-1.160)؛ عند مستوى معنوية <0.05*]. قد تكون النساء اللاتي لديهن "G" أليل في المورثة *ADIPOQ* rs2241766 T/G أكثر عرضة للإصابة بالسكري أثناء الحمل بسبب انخفاض مستويات الأديبونيكتين لديهن، مما قد يساهم في ضعف حساسية الأنسولين أثناء الحمل بغض النظر عن مؤشر كتلة الجسم.

الكلمات المفتاحية: أديبوكينات، السُمنة، ضعف إمتصاصية السكر، فرط شحميات الدم، هايبوادبيونكتينيميا.