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Association between adiponectin and *ADIPOQ (rs 1501299)* gene polymorphism in gestational diabetes mellitus patients

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ABSTRACT

Introduction: In this study, we seek to determine the association between the polymorphism of the *ADIPOQ (rs1501299)* gene and adiponectin levels in gestational diabetes mellitus (GDM) patients.

Methods: The study participants included 200 pregnant women, 100 healthy subjects as the control group, and 100 having GDM as the case group. The enzyme-linked immunosorbent assay (ELISA) was used to examine adiponectin levels. The polymerase chain reaction-restriction length polymorphism (PCR-RFLP) was also used for genotyping examination.

Results: Findings showed that GDM patients had significantly lower serum adiponectin levels (p=0.001). The T/T genotype homozygotes prevalence for rs1501299 polymorphism was significantly lower in GDM patients. (p=0.047).

Conclusion: Low serum adiponectin levels are associated with GDM. The T allele of the rs1501299 (+276 G/T) polymorphism of the ADIPOQ gene protects against GDM.

Keywords: gestational diabetes mellitus, adiponectin, polymorphism

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a prevalent pathological condition during pregnancy causing glucose intolerance. Although there are differences between the populations, their rates range from 1% to 14% in pregnant women. Women having GDM are exposed to perinatal complications and type 2 diabetes mellitus (T2DM). Offspring born to GDM mothers are exposed to early-onset obesity and glucose intolerance. The mechanisms responsible for GDM are not fully understood. Studies in recent years reported the possibility of regulation of insulin resistance via adipocytokines during pregnancy and the increased GDM risk in women with low adiponectin levels. GDM during pregnancy increases the risk of preeclampsia, fetal overgrowth (macrosomia), neonatal hypoglycemia, and perinatal mortality [1-4].

Adiponectin, which is associated with insulin resistance, is an adipocyte-derived protein. Adiponectin, as a polypeptide hormone, is physiologically active and has positive effects by enhancing the activity of protein kinase (AMPK), inhibiting gluconeogenic enzymes, and reducing glucose concentration [5]. It constitutes 0.01% of the total circulating plasma proteins with a plasma level ranging from 3 to 30 µg/mL [6]. Rentankaran et al. have displayed that pregnant women with GDM had decreased adiponectin concentration [7]. In some studies, an inverse relationship has been reported between low levels of adiponectin and insulin resistance [8]. The adiponectin gene ***(ADIPOQ),*** an important regulator of adiponectin production and secretion, is located on the 3q27 chromosome. G to T substitution in intron 2 (***rs1501299***, +276G > T) is one of the commonly studied SNPs at the ***ADIPOQ*** locus [9]. Alfaigh et al. have reported that the ***ADIPOQ*** gene ***rs1501299*** gene's polymorphism was related to serum adiponectin and reduced the prediabetes risk [10]. Despite the contradictory relationship between this SNP and insulin resistance, there are limited studies.

The present study investigates the association between ***ADIPOQ (rs1501299)*** gene polymorphism and adiponectin levels in GDM patients. To our knowledge, this study is the first study that assessed ***ADIPOQ (rs1501299)*** gene polymorphism and adiponectin levels in GDM patients in the Turkish population.

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METHODS

Study design

The present study has a case-control design and is conducted in the Department of Obstetrics and Gynecology, Çanakkale Onsekiz Mart University (COMU) Hospital, Çanakkale, Turkey. Of 200 pregnant women included in the study, 100 were healthy control, and 100 were GDM patients admitted to COMU Hospital between January 2019 and December 2021. The diagnosis of GDM was according to the 2010 IADPSG guideline [11]. Exclusion criteria included chronic systemic diseases, preexisting diabetes (type 2 or type 1), treatment with hypoglycemics or insulin, multiple pregnancies, alcoholism, preeclampsia, smoking, and supplements or drugs **(Fig. 1.).**

All the procedures were conducted based on the principles of the Helsinki Declaration. The Institute Human Ethics Committee approval (No: 2011-KAEK-27/2019-E.1900051725). All study subjects gave written informed consent before participation in the study.

DNA Extraction and Genotyping

A venous blood sample was collected from the antecubital vein and replaced in a tube containing genomic DNA, and EDTA was taken from blood samples with AccuPrep Genomic DNA extraction kits (Daejeon, Bioneer, South Korea). A spectrophotometer was used to assess DNA quantity and purity by absorbance values (Thermo Scientific, DeNovix DS-11+, USA). Genotyping for ***ADIPOQ*** polymorphisms ***(rs1501299)*** variants was conducted with Bioneer PCR with a final volume of 50 µL reaction and analyzed with agarose gel electrophoresis. The forward and reverse primers were 5'TGACCAGGAAACCACGACTC3' and 5'CCATCTACACTCATCCTTGG3', respectively. We performed PCR in a total volume of 50 µL, 100 ng of genomic DNA, 1× PCR master mix, and 100 picomoles of the reverse and forward primers. The specific reaction conditions are as follows: 55°C for 30 sec, 72°C for 1 min, 95°C for 10 min, extension at 72°C for 5 min, 95°C for 30 sec, and a total of 35 cycles. Finally, the PCR products' enzyme digestion was performed with the restriction endonuclease BsmI. Digestion of the PCR product by BsmI

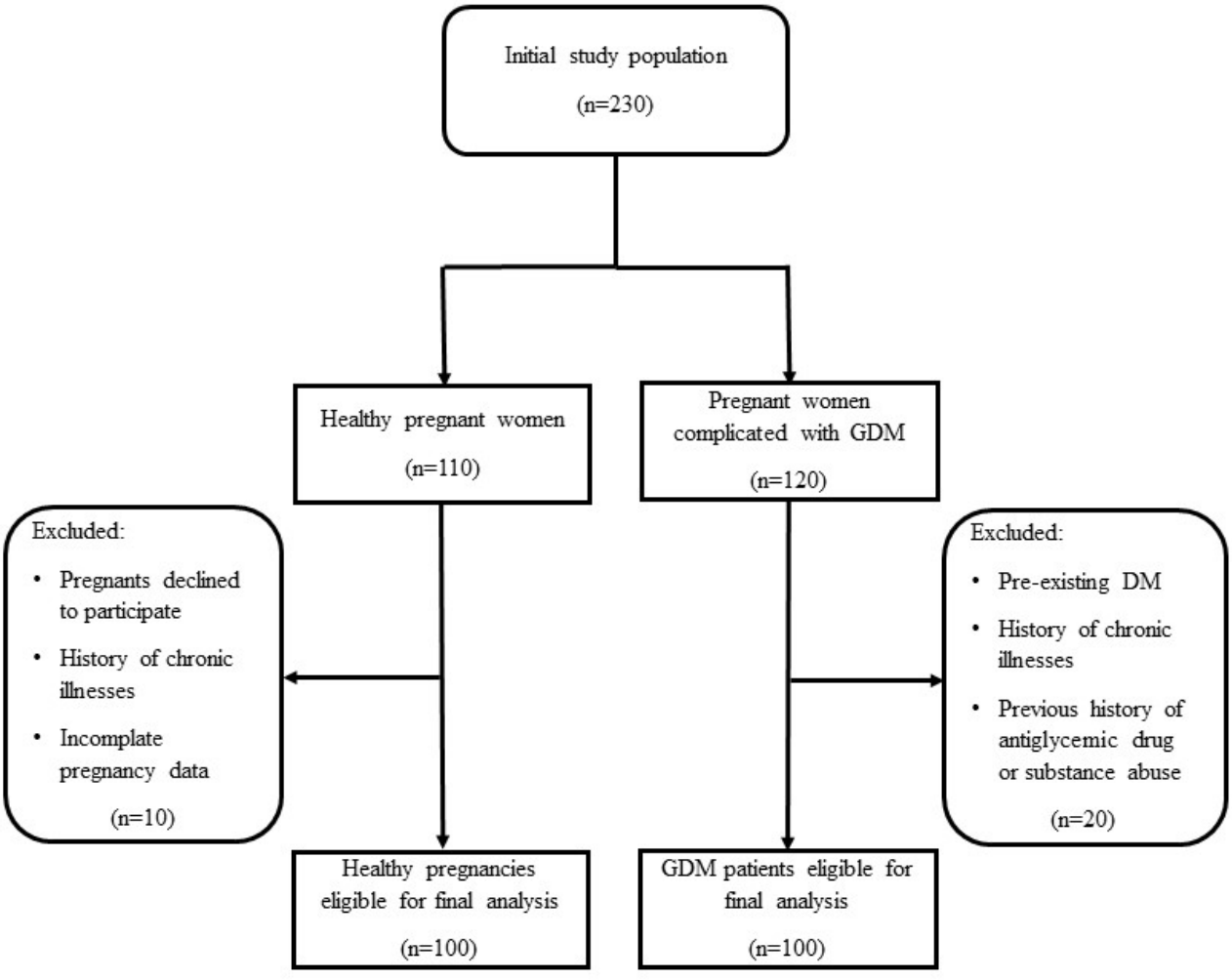

Fig. 1. Flow diagram of inclusion procedure of gestational diabetes mellitus (GDM) patients

yields 229/112 bp for GG, 341/229/112 bp for GT, and 341 bp bands for TT genotypes.

Adiponectin Measurements

Serum Adiponectin levels were measured with the ELISA method in both groups (Adiponectin Human Elisa Kit, Thermofisher, Waltham, MA). An antibody specific for human Adiponectin coated on a 96-well plate was used in this test. The ELx800 microplate reader (Allsheng Amr100 Instruments, Chinese) at 450 nm was used to measure absorbance.

Statistical analysis

We used SPSS statistical program for experimental data analysis. (version 19.0, SPSS Inc., Chicago, IL, USA). The consistency of the genotype distribution with Hardy-Weinberg equilibrium (HWE) was assessed using the exact test. We performed One-Way ANOVA and non-continuous variables $\chi 2$ test to test continuous variables' differences. All the analyses indicate $p<0.05$ as a significant difference.

RESULTS

The patients' demographic and biochemical characteristics with groups' genetic analysis are shown in **Table 1.**

The mean age of GDM patients and the control group was 29.69±6.14 and 29.10±5.64, respectively (p=0.480). The mean gestational age for the GDM groups was 27.70±5.03 weeks (p=0.037). The BMI of GDM patients was significantly higher compared to the control group (p=0.050). The one–hour OGTT, HbA1c concentrations, fasting plasma glucose, and two-hour OGTT of the patient group were significantly higher than the control group (p=0.001).

In this study, the genotype distributions of all groups were found consistent with Hardy-Weinberg equilibrium (Chi squared = 0.757; p=0.685),. In the control group, G/G genotype was found in 52 (52%), G/T genotype in 38 (38%), and T/T genotype in 10 (10%) cases. When the patient group with GDM was analyzed, G/G genotype was found in 52 (52%), G/T genotype in 46 (46%), and T/T genotype in 2 (2%) cases (p=0.037).

The mean adiponectin level in the control group was 7.85±0.75 ng/mL. Serum adiponectin levels were significantly lower in the GDM group (4.85±0.47 ng/mL, p=0.001). The association between low adiponectin levels and GDM was shown in our analysis (p=0.001; 95% CI, OR, 0.44; 0.16-0.51). Furthermore, the rs1501299 GT genotype increased the GDM risk compared to the TT genotype (p=0.044; OR, 4.86; 95% CI, 0.42-0.95). A similar result was found for rs1501299 for the GG genotype, and it was determined that it increased the GDM risk compared to the TT genotype (p=0.025; OR, 4.93; 95% CI, 0.34-0.80) **(Table 2).** TT+GT/GG genetic models showed that the rs1501299 poly-morphism was not associated with GDM **(Table 3).**

Table 1. Demographic and genetic parameters in gestational diabetes mellitus patient and control groups

		GDM (n=100)	Control (n=100)	P value[a]
Age (mean±SD)		29.69±6.14	29.10±5.64	0.480
Gestational age (weeks)		27.70±5.03	22.04±5.64	0.037
Body mass index before pregnancy (kg/m^2)		29.83±4.43	26.00±4.36	0.050
Family history of diabetes (%)		17.0 (17.0)	15.0 (15.0)	0.424
Previous pregnancy with GDM				
	First Pregnancy (%)	37.0 (37.0)	26.0 (26.0)	0.102
	Yes (%)	17.0 (17.0)	13.0 (13.0)	
	No (%)	46.0 (46.0)	61.0 (61.0)	
Fasting plasma glocose (mg/dL)		94.30±10.50	84.36±7.28	0.001
Glucose at 60 min (mg/dL)		174.04±3.42	117.80±3.66	0.001
Glucose at 120 min (mg/dL)		137.59±4.07	108.54±2.70	0.001
HbA1C (%)		5.44±0.44	4.84±0.48	0.001
Total cholesterol (mmol/L)		245.96±4.15	222.13±4.15	0.110
Triglyceride (mmol/L)		252.53±12.14	169.19±7.85	0.001
High-density lipoprotein (mmol/L)		72.79±1.96	74.93±1.68	0.409
Low density lipoprotein (mmol/L)		163.58±4.01	143.59±4.02	0.001
Adiponectin (ng/mL)		4.85±0.47	7.85±0.75	0.001
rs1501299	G/G	52 (52.0%)	52 (52.0%)	0.047
	G/T	46 (46.0%)	38 (38.0%)	
	T/T	2 (2.0%)	10 (10.0%)	

[a] student's t-test for continuous variables (variables with normal distribution), Mann–Whitney U-test (variables that were not normally distributed).

Table 2. Serum adiponectin and rs1501299 polymorphism in gestational diabetes mellitus patients

	Odds ratio	P-value
Age (mean±SD)	0.78 (1.05-2.23)	0.480
Body mass index before pregnancy (kg/m^2)	0.42(2.59-5.05)	0.001
HbA1C (%)	0.91(0.91-6.04)	0.17
Total cholesterol (mmol/L)	0.99(0.99-1.02)	0.35
Triglyceride (mmol/L)	1.01(0.99-1.08)	0.14
High-density lipoprotein (mmol/L)	1.01(0.96-1.05)	0.58
Low density lipoprotein (mmol/L)	0.99(0.98-1.01)	0.35
Adiponectin (ng/mL)	0.44 (0.16-0.51)	0.001
rs1501299 T/T	1	
G/T	4.86 (0.42-0.95)	0.044
G/G	4.93 (0.34-0.80)	0.025

Table 3. Adiponectin rs1501299 polymorphism (GG and GT+TT) in gestational diabetes mellitus patients

	Odds ratio	P-value
Age (mean±SD)	1.05 (0.98-1.12)	0.820
Body mass index before pregnancy (kg/m^2)	0.94(0.86-1.03)	0.19
HbA1C (%)	0.65(0.22-1.89)	0.42
Total cholesterol (mmol/L)	0.99(0.96-1.02)	0.66
Triglyceride (mmol/L)	0.94(0.99-1.01)	0.94
High-density lipoprotein (mmol/L)	1.01(0.97-1.03)	0.77
Low density lipoprotein (mmol/L)	1.01(0.99-1.04)	0.18
Adiponectin (ng/mL)	0.81 (2.6-9.8)	0.37
rs1501299		0.57
TT+GT	1	
GG	0.08 (0.14-0.51)	

DISCUSSION

GDM is regarded as a metabolic disease in pregnancy. Although the pathophysiology of GDM is unknown, several factors can contribute to GDM, such as adipokine levels, inflammation, and oxidative stress [10, 12-15]. Adiponectin secreted by adipose tissue is a polypeptide cytokine encoded by the ADIPOQ gene. Some authors have indicated adiponectin's multifactorial role in metabolic syndrome, obesity, and GDM. Irregular adiponectin concentrations have been related to several reproductive diseases, including GDM [16-19].

The adiponectin gene's ***rs1501299*** polymorphism at position 276 (SNP276) has a relationship with T2DM pathogenesis and insulin resistance. As a result of the meta-analysis study of the polymorphism of rs1501299, there was a significant correlation between the adiponectin gene's SNP genotype TT + 276 G > T susceptibility to T2D and polymorphism in all populations included in the study [20]. In our study, the GG genotype was found at a similar rate, but the patient group had a lower TT genotype than the control group. These study results agree with the prior studies.

A genetic study found that in comparison of the G/G genotype with the T/T genotype, a higher index of insulin resistance, lower plasma adiponectin, and a higher T2DM developing risk were found in subjects with the GG genotype. Similar findings were seen in studies conducted in various ethnic groups in the association between rs1501299 gene polymorphism, insulin resistance, and T2DM susceptibility. It has been reported in pregnant women with obesity or GDM with low adiponectin levels associated with larger infants with high-fat mass [18, 21-23]. Alfagih et al. showed that prediabetes patients had lower serum adiponectin levels than the controls in polycystic ovarian patients (p=0.006) [10]. Tangjittipokin et al. showed that measurements of serum adiponectin levels tended to decrease in GDM compared with controls [24]. The present study found significantly lower levels of serum adiponectin in patients with GDM compared to the control group (4.85±0.47 ng/mL, p = 0.001). This result supports the association between low adiponectin levels and the development of GDM. These results are in agreement with prior studies.

One of the strengths of this study was that adiponectin concentrations were significantly reduced in GDM pregnant patients compared to the control group. GDM patients with alleles of ADIPOQ T (rs1501299) had lower levels of adiponectin. These results may play a role in risk estimation as a biomarker in the early diagnosis of the disease and the prevention of high glucose during pregnancy.

There are limitations to our study. The present study included a relatively small number of cases, due to the difficulties faced in selecting participants who met all of the established criteria. Therefore, the study is insufficient to talk about pregnancy complications. In addition, despite the use of a one-step test in our study for GDM diagnosis, the two-step test was used for the same purpose in similar studies in the literature.

CONCLUSIONS

In conclusion, changes in adiponectin expression level, SNPs in the adiponectin gene, and plasma concentration have an association with GDM. Although GDM is a multifactorial disease, the adiponectin gene's rs1501299 polymorphism could be associated with the pathogenesis of GDM. Considering the presence of several SNPs, not a single mutation in the adiponectin gene, studies involving large populations of different ethnic groups are needed depending on the patients' environmental factors, genetics, and lifestyle.

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AUTHORS' CONTRIBUTION

SC- Conceptualization, investigation, drafting the article

SU- Investigation and resources

FB- Methodology, validation, critical review of the article

EO- Drafting the article

ESP- Drafting the article

CONFLICT OF INTEREST

None to declare.

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