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Association between *TCF7L2* Gene Polymorphism (rs4506565) and with the Risk of Gestational Diabetes Mellitus in Samples of Iraqi Women

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Abstract: Gestational diabetes mellitus (GDM) a type of diabetes mellitus 2, a glucose tolerance disorder, first diagnosed during pregnancy especially after the first trimester, in women who have never been diagnosed with diabetes. *TCF7L2*-related SNPs (4506565) whose type 2 diabetes-associated risk alleles were also associated with a higher risk of GDM.This study aimed to highlighted association between *TCF7L2* gene polymorphism (rs4506565) and increased risk of gestational diabetes mellitus in samples of Iraqi women and found the relationship between this SNP and some serum Biochemical levels. The age range in both patient and healthy groups was 17 to 49 years. This study carried out at Institute of Genetic Engineering and Biotechnology, University of Baghdad, the samples were collected from laboratories of Yarmouk Hospital from November of 2022 to February of 2023. Genotyping of rs4506565 was done by HRM technology. The biochemical test of (FBS, HbA1c, ALT, AST, ALP) was determined automatically by COBAS C111 analyser System. The results of genotypes and alleles frequencies of *TCF7L2*gene (rs4506565) SNP, in controls *versus* Iraqi Women with GDM showed that the Heterozygous genotype AG and the Homozygous genotype mutant GG at (P=0.0005**) in patient in comparison to control. In addition, allele frequency G was statistically significant difference in women with GDM and without GDM groups at (P=0.001**). The results of Biochemical parameters indicated that increase in the mean of serum FBS and HbA1c concentration in the GDM than the control group with highly significant differences at (P=0.001). The results showed that the association between ALT, AST and ALP and risk of GDM, were non-significant at p-value (0.2, 0.09 and 0.2) respectively. The results also revealed that the relationship between (FBS, HbA1c, ALT, AST, ALP): and the genetic variant (rs4506565): the differences were non-significant with all alleles for patients and controls. The conclusion of this study found The GG and AG genotype may represent a risk factor against the incidence of patients with GDM in Iraqi pregnant women.

Keywords: Gestational diabetes mellitus, *TCF7L2* gene, polymorphism. FBS, HbA1c

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Introduction

Gestational diabetes mellitus is diabetes first diagnosed in pregnancy. It is a temporary rising of blood sugar levels in a pregnant female with no history of diabetes before gestational, it caused by not enough insulin. More than 21 million births are affected by maternal diabetes worldwide each year. GDM, together with its short- and long-term negative outcomes, is increasing in incidence all over the world (1). The current diagnostic method for GDM, the oral glucose tolerance test (OGTT): is dated and has been reported as inconvenient for women as well as poorly reproducible and reliable ,Nevertheless, High blood sugar can induce serious health problems in gestational diabetes, particularly, heart disease and nerve and kidney damage(2). The most frequently

measured indicator of liver disease is raised serum enzyme activity of aminotransferases such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and occurs mostly in diabetics than in healthy populations. Excessive hepatic glucose output in gestational diabetes pays to fasting hyperglycemia. The glomerular filtration rate (GFR) increases by ~50 percent during pregnancy (2). It was found that one of the genes responsible for the occurrence of gestational diabetes is the *TCF7L2* gene (3): which spans a 215863 base-pair region on chromosome 10q25.3, and this gene, in turn, encodes a transcription factor that plays an important role in the Wnt signaling pathway. In this pathway TCF7L2 protein induces transcription of genes involved in glucose homeostasis, such as intestinal proglucagon (3).

The rs4506565 variant of the *TCF7L2* gene have been identified to be significantly associated with the risk of GDM, which offers the potential to improve understanding of the etiology of GDM and especially the biological mechanisms related to beta cell function(3).

Materials and methods

The research was carried out in the laboratories of the Institute of Genetic Engineering and Biotechnology for postgraduate Studies at the University of Baghdad, private external laboratories, and in the laboratories of Yarmouk Hospital. The study Blood samples were collected from November 2022 to February 2023.The total samples collected were 100 samples from all pregnant women (with gestational diabetes and without gestational diabetes). It was divided into two groups that included pregnant women aged between 18-46 years.

Five milliliters was withdrawn from each volunteer using the usual single-use syringe, and this amount was divided directly into two parts: the first part, 2 ml, was transferred directly into a sterile Ethylene Diamine Tetra Acetic Acid (EDTA) tube and stored at (-20 ° C) for the purpose of conducting molecular tests.

The second part (3 milliliters) was transferred to a gel tube then it was left to clot at room temperature (20-25˚C) for 10 minutes, then centrifuged at 3000 rpm for 10 minutes to obtain blood serum that was divided into (0.5 ml) Eppendorf tubes and stored at (-20˚C) for biochemical tests.

Genomic DNA was extracted from whole blood from all pregnant women (with gestational diabetes and without gestational diabetes) using wizard genomic DNA purification kit (genomic DNA KIT EE121). Then, the extracted DNA was used for amplification of targeted fragments by using PCR. Specific Primers used in the study were designed according to their reference sequence in the database of National Center for Biotechnology Information (NCBI). All primers were supplied by Alpha DNA Company as a lyophilized product of different picomols concentrations. The sequences of these primers are listed in (Table 1).

Table (1): Primer of TCF7L2 rs4506565 A>G

Primer	Sequence (5′→3′ direction)	Product Pb
Forward	GGATGAGCCTCTCGGGATATG	20
Reverse	GCAGCATCTTTTCTTGATGC	20

Company origin: Alpha DNA- Canada

The PCR condition for amplification of SNPs fragment was as, 40 cycles of denaturation at 94 C, for 5 sec.; annealing at 56 C for 15 sec and

extension at 72 C for 20 sec.The biochemical test of FBS, HbA1c, ALT, AST, ALP was determined automatically by COBAS C111 analyzer System.

The Statistical Analysis IBM SPSS Statistics 26 program was used to detect the effect of different factors on study parameters. One-way ANOVA and T-test was used to significantly compare between means. Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability). Estimate of Odd ratio and CI in this study. GraphPad Prism 9 program was used to draw the figures in this study. WINPEPI and SPSS program was used to detect the genotyping (4).

Results and discussion

A total of 100 blood samples of pregnant women enrolled volunteers were collected and divided into two groups, 50 pregnant women with GDM and 50 pregnant individual controls who are apparently healthy. Biochemical markers (FBS, HbA1c, ALT, AST, ALP) tests.

Biochemical parameters

One of the main parameters of this study were to assess differences in the amount of glucose (FBS and HbA1c) between the patients and the healthy controls. The levels of FBS were measured in the blood serum of all samples, mean of serum FBS concentration in the patient group was (112.00±36.61 mg/dl) while there was an increase in the mean of serum FBS concentration in the GDM than in control group which was (91.31±12.31 mg/dl). The result of FBS indicated that the levels were significantly higher (P=0.001) in GDM patients in contrast with healthy controls, is shown in the (Table 2).

Table (2): Comparison between patients and control groups in FBS and HbA1C.

Groups of TCF7L2 gene (rs4505454)		FBS	HbA1C
Patients	Mean	112.00	6.10
	Std. Deviation	36.61	1.10
Control	Mean	91.31	5.10
	Std. Deviation	12.31	5.00
p-value		0.001**	0.001**

One of the symptoms of GDM is the change in biochemical markers including serum glucose, serum HbA1c, and leukocyte count (5). Alkaline phosphatase, inadequate pancreatic ß-cell response to increased insulin requirements during fetal development within physiological, pregnancy - associated insulin resistance, is implicated in the pathophysiology of this common pregnancy complication (6). According to the previous findings, FBS levels must be maintained within normal levels during the whole the gestation period to avoid negative pregnancy outcomes. An abnormal FBS level is considered a major diagnostic marker for GDM; furthermore FBS is simple, relatively inexpensive, reproducible test (1). Moreover, studies have found that examining the FBS alone can diagnose 50% of pregnant women that have been detected with DM using another screening method (7).The mean value of HbA1c was obviously higher in GDM (6.01±1.10 mg/dl) comparing with normal pregnant (5.10±5.00 mg/dl): the results revealed that there were extremely highly significant differences between the patients and healthy controls women (P=0.001) as shown in (Table 2). This result agrees with the previous studies which proved that concentration of HbA1c was higher in GDM (8).

Kulshreshtha *et al*. (9) indicated that HbA1c can be relied upon as a Predictor in Early Diagnosis of Gestational Diabetes Mellitus and showed the HbA1c can significantly diagnose GDM with high sensitivity and specificity and is therefore consistent with the result of this study. Also, results have been agreed with Almawla's (10) results, which showed that FBS and HbA1c were significantly higher in GDM Iraqi patients compared with control.Table (3) shows a comparison of the activity of liver enzymes AST, ALT, and ALP in the blood of pregnant women (Patient and healthy) with the risk of developing gestational diabetes.The results showed that the association between ALT, AST and ALP and risk of GDM, were non-significant at p-value (0.2, 0.09 and 0.2) *respectively*.

Table (3): Comparison between patients and control groups according to Liver function

Groups		ALT	AST	ALP
Patients	**Mean**	14.14	19.56	152.38
	Std. Deviation	11.22	11.67	96.05
Control	**Mean**	12.00	16.67	132.14
	Std. Deviation	6.60	4.60	86.80
p-value		0.2	0.09	0.2

The liver is a vital organ in metabolism that plays an important role in the regulation of glucose homeostasis. The markers for liver dysfunction, such as alanine AST and AST have been shown as a good indicator to measure the liver health and involved with hepatic insulin resistance, hepatic enzymes could be underlying biological markers connecting between liver disease and T2D, The AST, ALT, and ALP are enzymes that indicate liver activity and are involved in biological processes (11,12).

These results are similar to other researches Zhao *et al.* (13): who found that the ALT and AST values did not have significant statistical differences. It is possible that the lack of association between these enzymes in those studies is due to relatively lower BMI of the study participants both before pregnancy and at time of liver enzyme test as well. It may also be explained by the lack of correlation between ALT or AST and inflammation. No significant correlation was found between AST or ALT levels and GLU levels on either time point and increased risk of GDM.

The study conducted by Mishra *et al.* (14) on 33 women with gestational diabetes and 25 pregnant women who did not suffer from gestational diabetes on liver enzymes showed that the activity of ALP and ALT was not significant and only the activity of AST was statistically significant compared with the group of normal pregnant. While the Iraqi study conducted by Hadi and Al-Hashemi (15): in Baghdad on gestational diabetes patients came with AST results that agree and the ALT results which was the opposite with the results of this study.

The ALT/AST an indicator has been linked to metabolic disorders and has been able to follow changes in insulin sensitivity and B-cell function. Several liver enzymes, including ALT, and AST, have been linked to T2DM in previous research. Nonetheless, the link between GDM and a variety of liver enzymes remained disputed. In most prior investigations, AST was found to be unrelated to GDM. The research of Zhao *et al.* (13) found that the associations between ALT and AST and the risk of GDM were not-significant at (OR 1.32, and OR 0.76, respectively): this is similar to the current study results.

The ALT and AST values remain unchanged during normal pregnancy; however, their ranges are changed, with a reduction in the upper end. This is a consequence of hemodilution occurring during pregnancy. When the ALP rises (up to 300%) it is placental origin (16).

A number of researchers have concluded that one of the reasons for the difference in the results of their studies may be that the hypothesized that fetal gender could also affect placental ALP levels and thereby causes different serum levels of total and placental ALP between female and male bearing pregnant women (17).

Molecular study

Distribution genotype and allele frequency at *TCF7L2* gene rs4506565 A>G SNPs

For this study, only SNPs from the *TCF7L2* gene that affect the protein coding sequence were selected. Gene polymorphism (rs4506565) has three genotypes, Homozygous AA as a wild type, GG as a homozygous mutant and Heterozygous mutant AG genotype. The frequency of all genotypes analyzed among women who had GDM compared with those who served as a normal pregnant.

The table (4) in this study showed that the genotype contrast of TCF7L2 (rs4506565) polymorphism as well as allele frequencies between patient women and control of pregnancy was detected. The results showed that the Homozygous AA genotype (Wild) as a reference was found in 11(22%) patients and 27(54%) in control, the Heterozygous genotype AG (mutant) was found as a highly frequency and statistically significant at (P=0.0005**) in patient in comparison to control 28(56%) and 19(38%): respectively. Moreover, the Heterozygous genotype mutant GG was found high significant at (P=0.005**) in patient women 11(22%) while in control was 4(8%). In addition, allele frequency was statistically significant difference in women with GDM and without GDM groups at (P=0.001**). There was a G-allele related risk factor.

On the other hand, odds ratio was calculated with AA genotype (Wild) (OR 1.00) serving as the basis for comparison.

The significant odds ratio for the Heterozygous genotype(mutant) AG was 3.6, while the odds ratio for the Heterozygous genotype GG was 6.7. In addition, the odds ratio for the allele frequencies A and G was (1.00 and 2.7): *respectively*.

Table (4): Genotype and allele frequencies *TCF7L2* gene rs4506565 A/G SNP between patient group and control group

Genotype rs4506565 A/G	Patient group NO.=50	Control group NO.=50	P-value	OR	CI 95%
AA	11 (22%)	27 (54%)	--	1.00	(Reference)
AG	28 (56%)	19 (38%)	0.0005**	3.6	1.4538 to 9.0002
GG	11 (22%)	4 (8%)	0.005**	6.7	1.7638 to 25.8320
Total	50 (100%)	50 (100%)	--	--	--
Allele Frequency					
A	0.50 (50)	0.73 (73)	--	1.00	(Reference)
G	0.50 (50)	0.27 (27)	0.001**	2.7	1.4981 to 4.8794

The *TCF7L2* gene (rs4506565) SNP is located in non-coding regions (intron 3) but it is not clear if this SNP, or a variant in strong linkage disequilibrium with it, play a role in

alternative splicing, gene expression, or protein structure (18).

The results of this study agreed with those of Zhang et al. (19) who found that TCF7L2 (rs4506565) was associated with an increased in higher risk of GDM at (P= 0.031 and OR = 2.31).

Abdullah and Ali. (20) Conducted a study in Iraq to reveal the association of the rs4506565 polymorphism with type 2 diabetes on a number of affected patients (100 patients, 100 healthy controls). The result of the study was that this polymorphism does not show a significant association in the genotypes in all genotypes.

In a previous study conducted by Muhammad, (21) on the Malaysian population, the association of the (rs4506565) polymorphism with Type 2 was the strongest association in the (rs4506565): and that people carrying the variant alleles of the (rs4506565) polymorphism had higher levels of HbA1c by (6.5%): which may lead to a more serious disease in these people (21). A number of studies that were conducted in the Arab world, such as Qatar, Lebanon, Saudi Arabia, and the United Arab Emirates (UAE): were carried out by researchers Khan et al. (22), *respectively*. All the results of these studies did not find any significant association of (rs4506565) SNP with the risk of infection with Type 2DM (P>0.05). It is not clear why this variant does not confer a predisposition to T2DM in the UAE population group, although effects of nearby genetic variants cannot be ignored. Furthermore, these *TCF7L2* SNPs are in the intronic noncoding region of the gene and as a result the mechanism by which they influence the TCF7L2 gene product and diabetes phenotype is still not clear (38).

The rs4506565 for *TCF7L2* gene variant has been associated with gestational diabetes and Type 2 in Asian and Caucasian populations. Studies reported that the genetic variant rs4506565 was significantly associated with gestational diabetes, showing 1.98 times the risk of developing gestational diabetes in Caucasian American mothers, It is Associated the accumulation of genetic and epigenetic changes in genes with insulin secretion , Previous studies have suggested genes that play important roles in several pathways like lipid, glucose metabolism, insulin secretion and insulin resistance the genetic variants of which are associated with the development of GDM (23). This polymorphism was chosen from a screen of 15 SNPs and are selected because their minor allele frequency (MAF) was >2% or because they were reported to be associated with type 2 diabetes in previous studies.

Table (5): Number and percentage frequencies of *TCF7L2* rs4506565 A/G genotypes and their Hardy-Weinberg equilibrium (HWE) in the Control group and patients.

	Patient group		Control group	
	Observed	**Expected**	**Observed**	**Expected**
Wild AA	11	12.500	27	26.645
Hetero AG	28	25.000	19	19.710
Mutant GG	11	12.500	4	3.645
Total	50	50	50	50
p-value	0.3		0.7	

The results of the table (5) appeared according to the Hardy Weinberg equilibrium to calculate the percentage of the effect of this mutation in Iraqi society among pregnant women and between both patients and normal

women show that it was of non-significant at p-value 0.3 and 0.7 respectively.

Different ethnic groups may have different relationships between TCF7L2 variants and GDM risk, which may be due to genetic differences, sample size, or participant selection criteria, as well as differences in growth environments, body types, and genetic background. Therefore, it's crucial to do continuing study and methodical analysis (24).

Relationship between *TCF7L2* gene polymorphism (rs4506565 A>G) and (FBS and HbA1C) levels

From the study table (6): it was appeared that the relationship between the glucose parameters (FBS and HbA1C): and the genetic variant (rs4506565): the differences were non-significant for both of them with all alleles for patients and controls, at (P=0.4 and 0.5) for the parameter FBS, respectively, and at (P=0.4 and 0.3) for the parameter HbA1C, *respectively*.

Table (6): Relationship between *TCF7L2* gene polymorphism (rs4506565) with glucose levels

Groups	rs4506565 A/G		FBS	HbA1C
GDM Patients	Wild AA	Mean	100.50	5.71
		Std. Deviation	21.20	0.82
	Hetero AG	Mean	116.40	6.18
		Std. Deviation	40.64	1.24
	Mutant GG	Mean	112.40	5.86
		Std. Deviation	38.20	0.83
	p-value		0.4	0.4
Control	Wild AA	Mean	92.30	5.11
		Std. Deviation	14.70	0.51
	Hetero AG	Mean	91.25	5.00
		Std. Deviation	9.20	0.40
	Mutant GG	Mean	85.00	5.40
		Std. Deviation	6.33	1.00
	p-value		0.5	0.3

The study of Zhang *et al*. (19) agreed with the results of this study when they found that there were no significant differences in FBS levels among genotype for (rs4506565) SNP (*P*> 0.05). The researcher added that carriers of the genetic form rs4506565 had a 3.51 times risk of developing gestational diabetes compared to those without risky genetic forms.

In the study conducted by Abdullah and Ali. (23) on Iraqi people with type 2 diabetes to reveal the association of the rs4506565 polymorphism with type 2 diabetes, they found high statistical significance glucose standards (FBS and HbA1C): between control patients and type 2 patients ($p < 0.001$)): and this differs from the results of the current study.

In another study of the rs4506565 genetic variant that included Type 2 patients found that fasting plasma glucose levels and glycated haemoglobin were significantly higher compared to controls (24). In postmenopausal carriers for rs4506565 SNP, higher glucose levels have been detected in individuals who suffer from obesity, as well as individuals who have high subcutaneous fat. They may be at risk of developing dyslipidaemia compared to people who have low subcutaneous fat. As our opinion the reasons for the current results may be the effect of this genetic variant on the success of the therapeutic response to

diabetes treatments, since all pregnant women with gestational diabetes had taken one of the known diabetes treatments. While in unaffected pregnant women, sugar levels are low in the first place because they do not suffer from dysfunction.

Relationship between TCF7L2 gene polymorphism (rs4506565 A>G) and the concentration of AST, ALT and ALP enzymes

Table (7) shows that the correlation was not statistically significant between the rs4506565 polymorphism and the functions of liver enzymes in the study population, as the values of (P=0.3 and 0.7) for the ALT enzyme, (P=0.4 and 0.2) for the AST enzyme, and (P=0.4 and 0.2) for the ALP enzyme were for patients and controls, *respectively*.*TCF7L2* has a clear vital role in various biological processes and functions in many tissues and organs, including liver tissue and adipose tissue. In addition, *TCF7L2* acts as a regulator of gluconeogenesis in the liver and promotes fat accumulation. *TCF7L2* in islets not only affects insulin secretion from beta cells, but it has an effect on other cells as well, as well as the formation of lipids in adipose tissue(25).

Table (7): the association between TCF7L2 gene polymorphism (rs4506565) and the liver function

Groups	rs4506565 A/G		ALT	AST	ALP
Patients	Wild AA	Mean	18.3455	23.6545	143.7636
		Std. Deviation	25.61333	19.65413	101.81130
	Hetero AG	Mean	14.1321	18.2393	143.0286
		Std. Deviation	7.14345	8.92231	91.28732
	Mutant GG	Mean	9.9545	18.8309	184.8273
		Std. Deviation	4.94072	5.92534	104.19952
	p-value		0.3	0.4	0.4
Control	Wild AA	Mean	11.2200	16.4081	122.1696
		Std. Deviation	6.74250	4.39826	77.71719
	Hetero AG	Mean	12.6194	17.5142	155.0000
		Std. Deviation	6.19486	4.91557	101.80317
	Mutant GG	Mean	12.8075	13.1350	90.8750
		Std. Deviation	8.82962	3.42042	45.60361
	p-value		0.7	0.2	0.2

Conclusion

The results also revealed that the relationship between (FBS, HbA1c, ALT, AST, ALP): and the genetic variant (rs4506565): the differences were non-significant with all alleles for patients and controls. The conclusion of this study found The GG and AG genotype may represent a risk factor against the incidence of patients with GDM in Iraqi pregnant women.

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