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Genetic variation of IRS1 gene in women with gestational diabetes mellitus in third trimester stage in Iraq

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Abstract

Gestational diabetes mellitus (GDM) is a growing health concern that usually appears during the second and third trimester stage of pregnancy and is characterized by carbohydrate intolerance of variable severity. The aim of the present study was to scrutinize the relationship between the G972R polymorphism of the insulin receptor substrate-1 (IRS-1) gene with GDM in the Iraqi female population. One hundred and twenty of blood samples taken from healthy women (control) and women with gestational diabetes mellitus in 3rd trimester stage of pregnancy, fasting blood glucose (FBG) and HbA1c% measured to diagnose GDM, lipid profile (cholesterol, triglyceride, HDL, LDL, and VLDL), insulin concentration, insulin resistance and beta cell function to determine risk factor for GDM, molecular study consist of DNA extraction and RFLP- PCR to study Genetic variation of IRS1gene in women with GDM. The fasting blood glucose mg/dl and HbA1c% level was increased highly significantly (P<0.01) between patient (GDM) and control (healthy women) in 3rd trimester stage in addition lipid profile included cholesterol mg/dl, triglyceride mg/dl, LDL mg/dl , VLDL mg/dl insulin concentration and insulin resistance but level of HDL mg/dl and beta cell function were decreased highly significantly (P<0.01) between patient (GDM) and control. Also the frequency of allele T was recorded a highly significantly (P<0.01) in patient (GDM) (0.87%) while in control(0.60%), the frequency of allele C allele significant (P<0.01) in control(0.40%) while (0.13%) in patient (GDM) in third trimester stage in pregnancy in third trimester. The results of this study it can be concluded that the genetic variation of IRS1 gene was associated with gestational diabetes mellitus comparison in control (healthy women) in Iraqi women in third trimester of pregnancy.

Keywords: gestational diabetes mellitus, polymorphism, gene, insulin, hyperglycemia

التغايرات الوراثيه لجين IRS1 في النساء الحوامل المصابات بسكري الحمل في العراق في مرحلة الحمل الثالثة

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الخلاصه

سكر الحمل هو حاله صحيه تظهر في المرحله الثانيه والثالثه من الحمل ويتميز بتغيرات حادة في تحمل الكاربوهيدرات. هدف الدراسه هو علاقه التغايرات الوراثيه لجين IRS1 مع سكري الحمل في المجتمع العراقي. شملت الدراسه مئه وعشرون عينه دم من نساء اصحاء ونساء مصابات بسكري الحمل. قيس كل من

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سكر الدم الصائم ، السكر التراكمي، والدهون وتشمل الكولستيرول TC ، الدهون الثلاثيه TG ، البروتينات الدهنيه ذات الكثافه الواطئه LDL ، البروتينات الدهنيه ذات الكثافه الواطئه جدا VLDL و البروتينات الدهنيه ذات الكثافه العاليه HDL. كما شملت الدراسه قياس تركيز الانسولين ومقاومه الانسولين ووظيفه الخلايا بيتا.

الدراسه الجزيئيه شملت استخلاص الدنا و استخدام تقنيه RFLP– PCR لدراسه التغاير الجيني. النتائج التي تم الحصول عليها شملت ارتفاعا معنويا ($P<0.01$) لكل من نسبه سكر الصائم ، السكر التراكمي، نسبه الكوليستيرول ، الدهون الثلاثيه، البروتينات الدهنيه الواطئه جدا و البروتينات الدهنيه العاليه. بينما انخفاضا معنويا بالنسبه الى البروتينات العاليه الكثافه في النساء الحوامل المصابات بسكري الحمل مقارنه بالنساء السليمات . كما اظهر النتائج ارتفاعا معنويا ($P<0.01$) في تركيز الانسولين ومقاومه الانسولين بينما انخفاضا معنويا في وظيفه خلايا بيتا. تكرار الاليل T اظهر ارتفاعا معنويا ($P<0.01$) في النساء المصابات (0.87%) بينما في النساء السليمات (0.60%) يستنتج من الدراسه وجود ارتباط بين التغاير في جين IRS1 وسكري الحمل بمرحله الحمل الثالثه.

Introduction

Gestational diabetes mellitus GDM is plain the same as several degree of glucose intolerance, identification throughout pregnancy [1] and it generally develops during the 2nd and 3rd trimester of the pregnancy [2].The prevalence of GDM, which affects 2–22% of all pregnancies, varies across populations (e.g., ethnic groups)[3]. Risk factors for GDM include obesity, advanced maternal age, family history of type 2 diabetes mellitus (T2DM), past history of GDM, previous adverse pregnancy outcomes, and belonging to a high-risk ethnic group [4].The genetic background of T2DMmay also be a factor in GDM because ample evidence has demonstrated the presence of T2DMin women with GDM. Epidemiological studies confirmed that the prevalence of GDM is in direct proportion to the prevalence of T2DM [5]. GDM and T2DM share a common genetic background, including glucose intolerance, insulin resistance, and impaired insulin secretion. However, association with similar risk factors and the genetic variants used to determine the risk of developingT2DMmight also be associated with the prevalence of GDM [6, 7].

The insulin receptor substrate- (IRS-) 1 gene, locate on chromosome 2q36, encodes a member of the IRS protein substrate family. IRS-1 is an endogenous substrate of the insulin receptor, plays a crucial role in the insulin signaling pathway, and is expressed in insulin-sensitive tissues. Several genetic polymorphisms of this gene and their effects on insulin action have already been identified [8]. A glycine-to arginine substitution in codon 972 (Gly972Arg) of the IRS-1gene (rs1801278) has been shown to be associated with a high prevalence of T2DM and GDM due to insulin resistance and impaired insulin secretion [6, 9]. The G972R polymorphism of IRS-1, which is located between two potential tyrosine phosphorylation sites involved in binding of the p85 subunit of PI-3 kinase, has previously been associated with T2DM[10].

Materials and methods

Protocols and Experimental design

The work of this study was performed in University of Baghdad, Collage of Science. The study sample included (120) Iraqi pregnant women in third trimester stage, the sample collected from Baghdad hospital and were divided into two main groups: the first included 60 healthy pregnant women as control group and the second included 60 pregnant women with gestational diabetes mellitus. The healthy women with normal blood glucose levels while patient women with rising in levels of blood glucose via HbA1c% (Boditech kit, korea) and fasting blood glucose test (biosystem kit, Spain).

Collection of Blood Sample

Samples were taken from pregnant women (patient and control) after (12-14) hours fasting via vein puncture by Hypodermic needle and 10ml syringe. Five ml of venous blood into tube containing EDTA as the anticoagulant for RNA extraction and 5ml put in tubes without anti–coagulant for biochemical tests.

Biochemical assays

Blood samples were left to clot for 20-30 minutes at 37C in an incubator. Serum was separated by centrifugation at 3000 rpm 10 minutes to measurement all biochemical tests which including: total serum cholesterol, triglyceride, LDL,VLDL, and HDL levels by use enzymatic method (Spinreact, spain). Serum concentrations of insulin were assessed using an ELISA kit specific for human insulin according to the manufacturer's instructions (DRG Instruments Mbh, Germany).

Molecular study

Total DNA was extracted from peripheral blood leukocytes using G-spin^{TM}Total DNA Extraction kit. DNA samples were stored at −70 ºC. In this study PCR was used to amplify and genotype the nucleotide 972 polymorphism (i.e., rs1801278) in IRS-1, which is responsible for the glycine-to-arginine amino acid mutation. Amplification of the fragment was performed with forward primer 5'-CTTCTGTCAGGTGTCCATCC - 3'and reverse primer 5'- TGGCGAGGTGTCCACGTAGC - 3'. Reaction condition of PCR summarized in Table-1

Table 1-Reaction condition of PCR for (rs1801278) IRS1 polymorphism

Stage	**Temperature (time)**	
Initial Denaturation	94°C (3 min)	
Denaturation	94°C (35 sec)	30 cycle
Annealing	61 °C (35 sec)	
Extension	72°C (35 sec)	
Extension	72°C (7 min)	
Hold	4°C	

The PCR product was electrophoreses on agarose gel and stained with red stain. The 299-bp product were digested for 25 min with BstNI (CC↓WGG) by PCR-RFLP. Reaction condition summarized in Table-1

Table 2-Reaction condition of Restriction Enzyme BstN1

Reagent	**Volume(µl)**
PCR product	5
Restriction Enzyme BstN1	1
Buffer	3
D.W.	1
Temperature/Time	60°**C** /25 min

The genotypes were determined by electrophoresis:G972 homozygotes (195/81/23 bp) and R972 (144/81/51/23 bp band).

Results

The result in this study showed at third trimester stage, the HbA1C was significantly increased (T test=77.90 P<0.01) in patients with GDM (8. 64 ± 0.72) when compared with control (5.34 ± 0.39).Also the level of fasting blood glucose FBG (mg/dl) was determined in patients with GDM and healthy group. A highly significantly (T-test= 7.45 P<0.01) in patients with GDM (198.08 ± 33.12) and control (96.36 ± 6.63)**.** Insulin concentration was highly significantly (T-test=4.76 P<0.01) in patients with GDM (39.70 ± 2.13) and control (23.91 ± 2.43), while Insulin resistance was highly significantly (T-test=33.82 P<0.01) in patients with GDM (15.28 ± 1.49) and control (7.49 ± 1.43) and B. cell function was significantly (T-test=15.943 P<0.05) in patients with GDM (26.83 ± 9.50) and control (49.99 ±11.17)Table-3.

Table 3-Levels of HbA1C, FBS, Insulin, Insulin Resistance and B. cell function in patients with GDM and control

Parameters	Mean ± SE		T-Test	P-value
	GDM	Control		
HbA1C (%)	8.64 ± 0.72	5.34 ± 0.39	77.90 **	0.0039
FBS (mg/dl)	198.08 ± 33.12	96.36 ± 6.63	7.45 **	0.0116
Insulin (ng/ml)	39.70 ± 2.13	23.91 ± 2.43	4.76 **	0.0012
Insulin resistance	15.28 ± 1.49	7.49 ± 1.43	33.82 **	0.0055
B. cell function	26.83 ± 9.50	49.99 ±11.17	15.943 *	0.0510
** (P<0.01), NS: Non-Significant.				

Also the study showed, the level of cholesterol (mg/dl) was determined in patients with GDM and healthy group. A highly significant (T-test=54.15P<0.01) in patients with GDM (252.80 ±21.35) compared with control (150.92 ± 9.76), also the level of triglyceride (mg/dl) was highly significant (T-test=69.61 P<0.01)in patients with GDM(250.87 ± 25.12) and healthy group(157.02 ± 16.73), while the level of LDL (mg/dl) was highly significant (T-test=40.98 P<0.01)in patients with GDM (220.54 ±10.91) and healthy group(122.19 ± 14.03), the level of HDL (mg/dl) was highly significant (T-test=19.72 P<0.01)in patients with GDM (32.48 ±3.32) and healthy group(67.16 ± 7.87) and the level of VLDL (mg/dl) was highly significant (T-test=14.35 P<0.01)in patients with GDM (45.11 ± 5.51) and healthy group(23.72 ± 3.41)Table-4.

Table 4-Lipid profile in patients with GDM and control

Parameters	Mean ± SE		T-Test	P-value
	GDM	Control		
Total cholesterol (mg/dl)	252.80 ±21.35	150.92 ± 9.76	54.15 **	0.0025
Triglyceride (mg/dl)	250.87 ± 25.12	157.02 ± 16.73	69.61 **	0.0114
LDL (mg/dl)	220.54 ±10.91	122.19 ± 14.03	40.98 **	0.0006
HDL (mg/dl)	32.48 ±3.32	67.16 ± 7.87	19.72 **	0.00387
VLDL (mg/dl)	45.11 ± 5.51	23.72 ± 3.41	14.35 **	0.0040
** (P<0.01).				

ISRI Gene

The PCR products were digested using restriction enzyme BstN1then RFLP PCR products were separated on agarose gel (Figure-1).

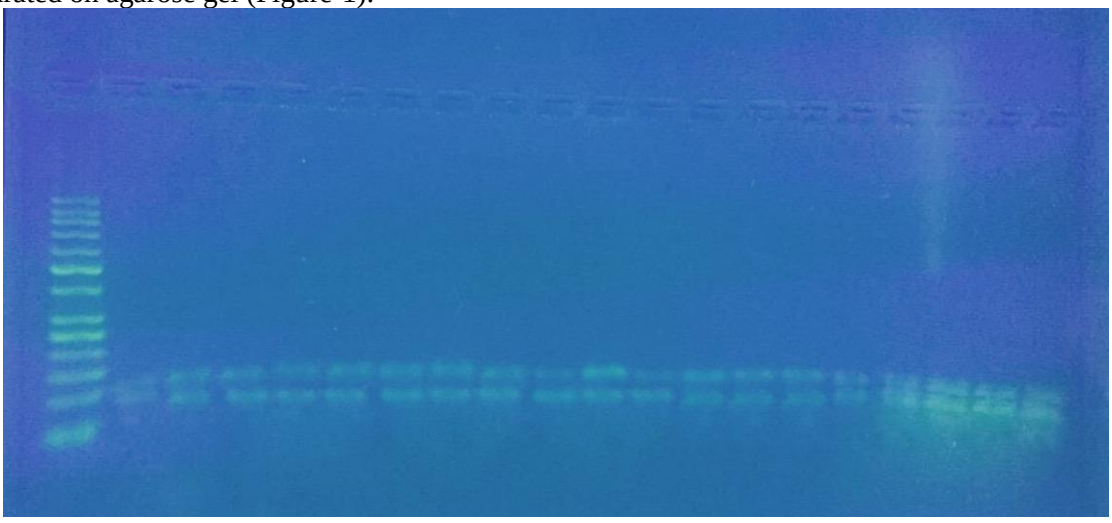

Figure 1-Electrophoresis pattern of PCR product digested with BstN1 restriction enzyme (2.5% agarose gel). Lane's 2, 17, 18, 19, 20 homozygous: CC genotype; Lane's 3.4.5.6.7.8.9.10.11.12.13.14.15.16 homozygous: TT genotype. M: molecular marker (100 bp DNA ladder), stained with red stain bands in the gel

The genotype variation of IRS1 (rs 1801278) polymorphism and allele frequency in control and patients with GDM at third trimester result revealed that the genotype TT showed significant differences (P-value = 0.0001 O.R. =1.526) in control (20.00%) while in patient with GDM (73.00%), genotype TC showed significant differences(P-value = 0.0001 O.R. =1.755) in control (0.00%) and in patient with GDM (0.00%), and genotype CC showed no significant differences(P-value = 1.00 O.R. =0.00) in control (80.00%) while in patient with GDM (16.67%). Moreover significant differences were noticed among three genotype (TT, TC, CC) in control (χ^2=13.00) and patient with GDM (χ^2 =11.083). In addition, the frequency of allele T was recorded a highly significance (P<0.01) in patient (GDM) (0.87%) while in control (0.60%), the frequency of allele C allele significant (P<0.01) in control (0.40%) while (0.13%) in patient (GDM) in third trimester stage in pregnancy Table-5.

Table 5-Genotype of the IRS1 (rs 1801278) polymorphism and the allele frequency in control and patients with GDM in third trimester of pregnancy

Genotype	Control	Patients	P-value	O.R.
	No. (%)	No. (%)		
TT	6 (20.00)	22 (73.33)	0.0001 **	1.526
TC	0(00.00)	0 (0.00)	0.0001 **	1.755
CC	24 (80.00)	8 (16.67)	1.00 NS	0.00
Total	30 (100)	30 (100)	---	---
Chi-square value (χ^2)	13.00 **	11.083 **	---	---
Allele frequency				
T	0.60	0.87	0.0001 **	---
C	0.40	0.13	0.0001 **	---
** (P<0.01).				

Discussion

At third trimester stage, the increasing in FBG level in patient with gestational diabetes agreement with many research that abnormal FBG level is a significant indicator in diagnosing GDM, it's category of irregular glucose tolerance throughout pregnancy [11]. The women with elevated HbA1c levels (<4.5% and ≥6.0%) had a advanced danger of undesirable pregnancy outcomes [12]. physiological condition of pregnancy is a characterized by a progressive gestation period reliant increase in level of triglycerides (hypertriglyceridemia) and level of cholesterol (hypercholesterolemia) [13, 14]. Previous study discovered that the glycated hemoglobin A1c (HbA1c) rank through pregnancy may be used as a test or diagnostic test for gestational diabetes (GDM) [15]. Earlier research have indicated that in early pregnancy and the first-trimester HbA1c level increasing may characterize a useful capacity to test women with GDM. on the other hand, in the 2nd -trimester and 3rd pregnancy [16].Result in this study showed that increased in insulin level in third trimester stage. Grissa *et al*., (2010) [17] suggested that the gestational diabetes mellitus pregnant women were hyperinsulinemic and hyperglycemic contrast with control that reflecting a reduce in insulin sensitivity in these persons, hyperinsulinemia could be responsible for high incidence of hyperglycemic mistakes in the newborns.

The pathophysiology of GDM is a role of reduced insulin sensitivity of mother or raised insulin resistance, also Noaemi and Shalayel, (2011)[18] suggested that the main reason of insulin resistance throughout gestational diabetes is post-cellular damage manifested by reduced tyrosine phosphorylation remains in receptors of insulin and insulin receptor substrate-1 whereas serine phosphorylation is raised which reduce signaling of insulin from activating GLUT4translocation. Buchanan and Xiang, (2005)[19] observed that a possible etiology for gestational diabetes mellitus is a limitation in β-cell function that apparents as hyperglycemia when secretion of insulin does not raise to equal the increased needs of insulin throughout pregnancy**.** Gestational diabetes mellitus is broadly related with endothelial dysfunction in the placenta chiefly triggered via hyperinsulinemia, hyperglycemia, and altered in concentration of nucleoside extracellular and dyslipidemia linked with this pathology could participate a task in this phenomenon since dyslipidemia is a risk factor to expand endothelial dysfunction and atherosclerosis [20]**.** Wilcox (2005)[21] suggested lipid abnormalities

associated with insulin resistance affect all lipid fractions, most important to high triglycerides level, VLDL, LDL cholesterol, low HDL cholesterol [22]. The results of this study revealed that the variant (rs 1801278) of the IRS-1gene is significantly associated with GDM. The previous studies (Fallucca *et al*., 2006 ;Pappa *et al.*, 2011; Alharbi *et al*., 2014)[23,24,25] indicate that the polymorphic allele T is considerably related with IRS-1 and may be complicated in the development of gestational diabetes.

In contrast, Shaat *et al*.,(2005)[26] suggest that did not detect any relationship between the (rs 1801278) polymorphism and gestational diabetes. In another study has been reported that the variant was related with higher fasting blood glucose (FBG) and levels of insulin in pregnant women with gestational diabetes [27]. Shaat *et al*., (2005)[26] concluded that protein levels of IRS-1 gene are reduced in the adipose tissue of Scandinavian obese pregnant women with gestational diabetes. Because of the essential function of the IRS1 gene in the signal transduction pathway, numerous studies have recommended the role of polymorphisms in IRS1 gene in the pathogenesis of type 2 diabetes and gestational diabetes [28].

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