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GENE EXPRESSION OF TCF7L2 IN WOMEN WITH GESTATIONAL DIABETES MELLITUS IN SECOND TRIMESTER

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ABSTRACT
Gestational diabetes mellitus (GDM) is elevated in any degree of blood glucose level, respect during first pregnancy and it evolves and continues during the second and third trimester of the pregnancy. One hundred and twenty blood samples collected from healthy women as control and women with gestational diabetes mellitus in second trimester stage of pregnancy, fasting blood glucose and HbA1c measured to diagnose GDM and lipid profile to determine risk factor for GDM, molecular study include RNA extraction and qRT- PCR to study gene expression of TCF7L2 in women with GDM. The level of fasting blood glucose FBG mg/dl and HbA1c % were increased highly significantly ($P<0.01$) in patient with GDM when compared with control in second trimester stage. Also the level of cholesterol mg/dl, triglyceride mg/dl, LDL mg/dl and VLDL mg/dl showed highly significant increase ($P<0.01$) in patient with GDM when compared with control in second trimester stage, while the level of HDL mg/dl decreased highly significantly ($P<0.01$). The result revealed high significant different fold change in expression of TCF7L2 gene between control (1.00 ±0.00) and patient with GDM (0.019 ±0.002) in second trimester. The low expression of TCF7L2 gene was associated with susceptibility of GDM comparison in control in Iraqi women during second trimester of pregnancy.

KEY WORDS: Gestational diabetes, lipid profile, TCF7L2, HbA1c, qRT- PCR.

INTRODUCTION

Gestational diabetes mellitus (GDM) is clear as any glucose fanaticism degree, respect during first pregnancy[1] and generally it evolves during the second and third trimester of the pregnancy[2]. It is the most rates contrast depending on population characteristics like age of mother, customs and body mass index BMI[3]. Many studies have shown that Asian women had upper risk of Gestational diabetes mellitus more than women of United States Caucasian or Australian origin[4]. Many other risk factors including, genetic disposition, polycystic ovary syndrome, hypertension and obesity have also been associated with the risk of GDM[5]. Gestational diabetes mellitus is related with undesirable maternal and neonatal outcomes [6,7] and excluding greater than before rate of Cesarean section delivery, macrosomia, and perinatal mortality[8]. Previous studies [9,10] indicated that a certain other conditions such as pre-eclampsia, polyhydramnios, hyperbilirubinemia, hypocalcemia, polycythemia, mental retardation, birth trauma and neonatal mortality. Autism spectrum disorder and long-term neuropsychiatric morbidity are associated with GDM [11].

MATERIALS & METHODS

Protocols and Experimental design

The protocol of this study was performed in University of Baghdad, Collage of Science. The study population included (120) Iraqi pregnant women in second trimester period and were divided into two main groups: 60 healthy pregnant women as control group and 60 pregnant women with gestational diabetes mellitus). The healthy women with normal blood glucose levels while patient women with increasing in blood glucose levels by using fasting blood glucose test (biosystem kit, Spain) and HbA1c % (Boditech kit, korea)

Collection of Blood Sample

Blood was drawn from pregnant women (patient and control) after (12-14) hours fasting via vein puncture by disposable syringes (10 ml). 5ml of venous blood into tube containing EDTA as the anticoagulant for RNA extraction and 5ml put in tubes without anti–coagulant biochemical tests.

Biochemical assays

Blood was left to clot for 20-30 minutes at 37°C in an incubator. Serum was separated by centrifugation at 3000 rpm 10 minutes to measurement all biochemical tests which including: total cholesterol, triglyceride LDL, VLDL, and HDL levels by use enzymatic method (Spinreact, spain)

Molecular study

RNA extraction by using (Direct-zol™ RNA MiniPrep, R2051), USA. To study gene expression of TCF7L2 used qRT-PCR technique that performed by use KAPA SYBR FAST one-step qRT-PCR kit, Canada. The primers used in study included:

TCF7L2 primer 5'GAAGGAGCGACAGCTTCATA-3' (Forward) 5'GGGGGAGGCGAATCTAGTAA- 3' (Reverse), Housekeeping gene primer HKG (α-tubulin) 5'-AGAGTCGCGCTGTAAGAAGC-3' (Forward) 5'- TGGT CTTGTCACTTGGCATC- 3' (Reverse). Expression of TCF7L2 gene using Smart cycler system and analyzed using Smart cycler® 2.0 software, the components and condition of reaction in Table (1 and 2).

TABLE 1: Component of the one-step RT-qPCR reaction

Component	20 µL (Final volume)	Final concentration
SYBR Fast qPCR master mix	10 µL	1 X
Forward primer (10µM)	0.5 µL	0.2 µM
Reverse primer (10µM)	0.5 µL	0.2 µM
Dntp	0.5 µL	200 nM
KAPA RT mix(Reverse transcriptase)	1 µL	1X
Tamplet RNA	6 µL	100 ng/µL
Distal water	1.5 µL	N/A

TABLE 2: Reaction conditions of the one-step RT-qPCR reaction

Step	Temperature	Duration	Cycles
Reverse transcription	42°C	5 minutes	Hold
Enzyme inactivation	95°C	3 minutes	Hold
Denaturation	95°C	3 seconds	
Annealing	59°C	20 seconds	40
Extension	72ºC	20 seconds	

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test or T-Test was used to significant compare between means.

RESULTS

At the second trimester stage of pregnancy, the level of fasting blood sugar was determined in patient with GDM and compared with healthy group. A highly significant increase (P<0.01) in patient (156.82 ±8.55) was found when compared with healthy group (90.85 ±3.62). Similarity the level of HbA1c % was highly significant increase (P<0.01) in patient (8.86 ± 0.55) when compared with healthy group (4.71 ± 0.22) table (3).

The result of lipid profile showed highly significant increase (P<0.01) in the level of cholesterol (255.79 ± 15.47 mg/dl), Triglyceride (230.57 ±20.67 mg/dl) , LDL (198.00 ±17.39 mg/dl) and VLDL (43.00 ±4.85 mg/dl) in patients with GDM when compared with healthy control (168.86 ± 7.61 mg/dl) ,(133.69 ± 4.83 mg/dl), (101.70 ± 10.75 mg/dl) and(25.70 ±2.01 mg/dl) respectively . The level of HDL was significant decrease (P<0.01) in patient (31.71 ±2.24 mg/dl) when compared with healthy control (67.29 ±3.53 mg/dl) table (3)

TABLE 3: Biochemical characteristic of study group

Tests	Control (mean ± SE)	Patient (mean ± SE)	T-Test
Fasting blood glucose mg/dl	90.85 ± 3.62	156.82 ± 8.55	19.512 **
HbA1C%	4.71 ± 0.22	8.86 ± 0.55	1.247 **
Cholesterol mg/dl	168.86 ± 7.61	255.79 ± 15.47	36.228 **
Triglyceride mg/dl	133.69 ± 4.83	230.57 ± 20.67	44.611 **
HDL mg/dl	67.29 ± 3.53	31.71 ± 2.24	8.806 **
LDL mg/dl	101.70 ± 10.75	198.00 ± 17.39	42.968 **
VLDL mg/dl	25.70 ± 2.01	43.00 ± 4.85	11.046 **
** (P<0.01)			

The results of molecular study showed high significant in fold change of expression of TCF7L2 in control (1.00 ± 0.00) while patient with gestational diabetes (0.019 ± 0.002) Table 4.

TABLE 4: Fold change in expression of TCF7L2 gene (control and patient GDM) in second trimester, calculated byΔΔ Ct method

Groups	Mean ± SE				
	CT	CT (HKG)	ct△	ct△△	Fold change
Control	21.57 ± 0.37	30.81 ± 0.62	-9.23 ± 1.06	0.00 ± 0.00	1.00 ± 0.00
Patient GDM	29.80 ± 0.59	30.79 ± 0.57	-3.47 ± 1.18	-5.95 ± 0.83	0.019 ± 0.002
T-Test	3.612 **	2.378 NS	4.063 **	2.275 **	0.366 **
P-value	0.0069	0.796	0.0001	0.0091	0.0084
** (P<0.01).					

Threshold cycle (ct) in Expression of TCF7L2 gene showed in figure 1

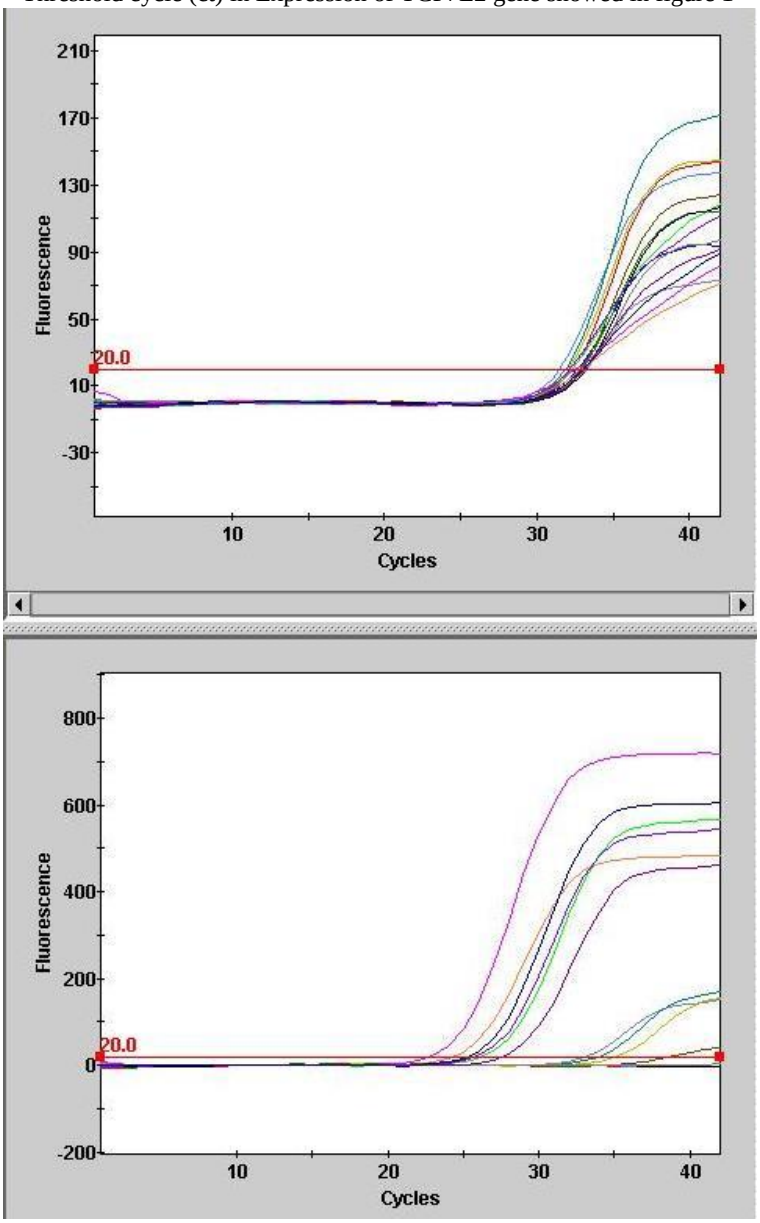

FIGURE 1: Expression of TCF7L2 gene using Smart cycler system and analyzed using Smart cycler® 2.0 software

DISCUSSION

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[12]. The women with elevated HbA1c levels (<4.5% and ≥6.0%) had an advanced danger of undesirable pregnancy outcomes[13]. physiological condition of pregnancy is a characterized by a progressive gestation period reliant increase in level of triglycerides (hypertriglyceridemia) and level of cholesterol (hypercholesterolemia) [14, 15]. It has been claimed [16] that the triglyceride levels elevations in during pregnancy in women with gestational diabetes compared with those lacking insulin resistance is essential as hypertrigly-ceridaemia is idea to be reason of fetal macrosomia. Wilcox [17] suggested lipid abnormalities associated with insulin resistance affect all lipid fractions, most important to high triglycerides level, VLDL, LDL cholesterol with low HDL cholesterol [18]. This result revealed that low expression of TCF7L2 in women with gestational diabetes compared with health women in second trimester stage of pregnancy. McCarthy *et al.* [19] has been observed down regulation of TCF7L2 gene expression, risk variants have been proposed to alter TCF7L2 splicing patterns, suggesting that functionally distinct mRNA isoforms, rather than levels of expression, define their phenotypic consequences. Extensive genome-wide association studies have revealed that specific single-nucleotide polymorphisms (SNPs) in TCF7L2 are strongly associated with the susceptibility of type 2 diabetes [20]. Because these risk SNPs are located within the intronic regions of TCF7L2, great effort has been made to assess whether these SNPs affect TCF7L2 transcription or its alternative

splicing [21]. The genome-wide association studies finding has also redirected our attention to the role of Wnt signaling and its downstream effectors, including TCF7L2 and β-catenin (β-cat), in controlling hormone--gene expression and glucose disposal[22]. Obviously, these contradictory observations were made because of the complexity of the Wnt signaling pathway. Because TCF7L2 has multiple alternatively spliced isoforms, it is possible that different isoforms may exert different or even opposite functions [23].

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