Original Research Article

Glucokinase Gene Mutations in Subjects with Gestational Diabetes Mellitus from Gaza Strip

Abstract

Objective: this study was conducted in order to evaluate the frequency of *GCK* gene mutations in exons 7, 8 & 9 in women with GDM and their relationship to some biochemical parameters as compared to healthy controls.
Methods: Samples were collected from 45 GDM women and 42 apparently healthy pregnant women. DNA was extracted and the samples were screened for *GCK* exons 7, 8 & 9 mutations at positions C.682A>G (p.Thr228Ala); C.895G>C (p.Gly299Arg) and C.1148C>A (p.Ser383X), respectively. The mutations were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methodology. Investigated biochemical features included: fasting blood glucose (FBG), oral glucose tolerance test (OGTT), HbA1c, insulin and the lipid profile.
Results: The results showed that 9 out of the 45 (i.e., 20%) GDM subjects harbored the exon 8 (895G>C) mutation. Neither exon 7 (c.682A>G) nor exon 9 (c.1148C>A) was encountered in the study population. Moreover, the level of FBG, OGTT and HBA1c were higher in the c.895G>C mutation-positive subjects, as compared to mutation-negative ones.
Conclusions: The screening of GDM patients for *GCK* gene mutations allowed for the identification of glucokinase-deficient patients diagnosed as GDM. Therefore, molecular screening is important for the differential diagnosis of GDM and MODY2 and consequently, proper patient management.

Keywords: GDM, Glucokinase, *GCK* Mutations, MODY2, Gaza Strip- Palestine

Running title: Glucokinase Gene Mutations in GDM

Introduction

Gestational Diabetes Mellitus (GDM) is defined as glucose intolerance resulting in hyperglycemia of variable severity with onset during pregnancy (Metzger et al., 1998). GDM occurs if pancreatic β-cells are unable to face the increased insulin demand during pregnancy (Buchanan and Xiang, 2005). Monogenic forms of diabetes resulting from mutations in single genes may also manifest during pregnancy. Polymorphisms in the promoter of *GCK* and polymorphisms of hepatocyte nuclear factor 1α (*HNF1α*) genes are common variants in MODY genes that increase the risk of GDM (Farrar, 2016).

Glucokinase, termed also glucose sensor, in pancreatic β-cells plays a crucial role in insulin secretion and regulation (Osbak et al., 2009). Heterozygous inactivating mutations in *GCK* are characterized by mild fasting hyperglycemia appearing at variable ages, while homozygous inactivating *GCK* mutations result in a more severe phenotype presenting at birth as permanent neonatal diabetes mellitus (Gloyn, 2003).

Prevalence of GDM varies widely worldwide, from 1% to 14% due to different ethnicities and the different diagnostic criteria used (American Diabetes Association, 2004). In the Arab world, a study implemented in the Gulf Cooperation Council (GCC) countries, reported variable rates in the prevalence of GDM; with 4.2 % in Oman, 10.1% in Bahrain, 16.3% in Qatar and 2.7 – 12.5 % in Saudi Arabia. In the same context, the prevalence of GDM in Egypt is around 8% (Khalil et al., 2017). According to a local study in Gaza-Palestine to identify the prevalence and sociodemographic characteristics of GDM in Gaza strip-Palestine, the prevalence of GDM was around 1.8% (Alkasseh and Aljeesh, 2014).

Adverse pregnancy outcomes of GDM are mainly related to macrosomia caused by fetal hyperinsulinism in response to high glucose levels coming from maternal hyperglycemia (Baz et al., 2016). Criteria of GDM diagnosis and screening recommendations have been recently updated. Patients at high risk should be early screened using FBG, and if the result is normal, at 24–28 weeks of gestation using OGTT (75 g) (Reece et al., 2009).

The definite diagnosis of MODY can be done by screening *GCK* mutations in patients which in turn helps in predicting the likely prognosis and clinical course. MODY mutations result in a feature phenotype regardless of the wide variety of mutations characterized by elevated FBG with the majority of the patients having blood glucose values within a tight range of 6–8 mmol/l (Kamata et al., 2004). Patients with *GCK* mutations have mild stable fasting hyperglycemia and barely have noticeable symptoms. Those patients are usually detected accidently by routine screening for medical purposes during pregnancy, or family screening when MODY is suspected (Baz B. et al., 2016).

The treatment lines in MODY-*GCK* are usually not needed as complications in these cases rarely appear. Therefore, the majority are managed by diet alone (Thanabalasingham and Owen, 2011). A recent study showed that the mean HbA1c was unaltered by discontinuing insulin or oral hypoglycemic agents in 87% of the GCK-MODY patients. Additionally, overtreatment with oral hypoglycemic agents or insulin therapy has been reported, and may be especially risky for patients with *GCK* mutations because they have an altered counterregulatory response to hypoglycemia (Carey et al., 2007).

Genetic studies have suggested that various disorders of glucose regulation would result from mutations in *GCK* gene (Osbak et al., 2009). More than 700 mutations have been reported in the *GCK* gene including, missense, nonsense, and frameshift mutations. Around 65% of the mutations are however, missense mutations (Gloyn, 2003).

Materials & Methods

Study population

Our study included 45 pregnant women with GDM, who were following at the primary health care centers across Gaza strip and having glucose intolerance measurements. In addition to 42 normoglycemic pregnant women who were age-matched with cases. The study was done at the Genetics Lab., Islamic University of Gaza.

Ethical approval

An approval to perform the study was taken from Helsinki Ethics Committee (PHRC/HC/240/17). Informed consent was taken from all women who accepted to participate in this study after well explanation of the procedures and objectives and considerations beyond the study.

Exclusion criteria

Exclusion criteria were patients with diabetes type 1 or type 2, aged under 24 or above 37 years old. Patients suffering from severe complications; liver disease, thyroid disorders or other endocrine disorders or chronic diseases. While inclusion criteria stand on second trimester gestation (24-28) weeks, mild hyperglycemia (92-126 mg/dl).

Questionnaire

A questionnaire was designed to match the study needs for both cases and controls. A meeting interview was done to fill in the questionnaire. The questionnaire included Scio-demographic data (e.g. age, gestation age, education). Anthropometric measurements such as (weight, height, BMI). Data concerning first degree family history of diabetes and gestational diabetes as well as systolic and diastolic blood pressure.

Blood samples collection, processing and analysis

Around 6 ml of blood were drawn from the (87) study participants; 45 of them diagnosed with GDM, and 42 as controls. 4 ml of collected blood were dispensed in 2 EDTA tubes for molecular analysis and determination of HbA1c. 2 ml in plain tube for the different biochemical analysis including FBG, insulin, triglycerides, cholesterol, HDL and LDL. The different biochemicals parameters were determined using commercial kits, LDL was calculated using the Friedewald formula and HOMA-IR was calculated using the formula (HOMA-IR = [fasting insulin (µU/ml) × fasting plasma glucose (mg/dl)]/405).

Molecular analysis

DNA was extracted from blood samples by using Wizard Genomic DNA purification Kit. Manufacturer instructions were followed. Exons (7, 8 and 9) of *GCK* gene were screened for the intended mutations using PCR-Restriction Fragment Polymorphism (PCR-RFLP) approach. The primers used for the PCR amplification of the required exonic fragments, amplicon size, restriction enzymes and size of digested products are presented in Table 1.

Table (1): PCR primers sequences, amplicon size, restriction enzymes, and the size of digestion products.

***GCK gene* mutation**	**PCR Primers (5`→3`)**	**Amplicon size (bp)**	**Restriction enzyme**	**Digested product (bp)**	**Ref.**
Exon 7 c.682 A>G	F: TGCAGCTCTCGCTGACAGTCC R: CTCCCATCTGCCGCTGCACC	**287**	*HhaI*	A allele 153 & 135 G allele 153, 93 & 42	[15,16]
Exon 8 c.895 G>C	F: CGTGCCTGCTGATGTAATGG R: GCCCTGAGACCACGTCTGC	**268**	*HhaI*	G allele 152 & 116 C allele (uncut) 268	
Exon 9 c.1148 C>A	F: CTGTCGGAGCGACACTCAG R: CCCCCAAATCTAGGCCAACC	**410**	*BfaI*	C allele 398 & 12 A allele 323, 75 & 12	

Statistical analysis

Data were computerized and analyzed using the Statistical program (SPSS/version 22.0). Different statistical tools were used. Chi-square (χ^2) was used for testing the significance of relations, associations and interactions between qualitative (nominal) variables. T-test (independent samples) was applied to examine whether there is statistically significant difference between the means of two unpaired samples. The one-way analysis of variant (ANOVA) was used to compare the means of independent groups. Pearson correlation test was applied to measure the strength and direction of linear relationship between two numerical variables. The results in all the previously mentioned statistical tools were considered as a significant when the P-value was less than 5% ($P<0.05$).

Results

PCR and PCR-RFLP products of exon 7

Figure 1 shows the PCR and PCR-RFLP products of exon 7 to detect p.Thr228Ala missense mutation at nucleotide 682 (A>G). The restriction enzyme (*HhaI*) cuts the mutant allele and yields three fragments (42, 93 & 153 bp), while the wild-type allele is digested into two fragments (135 & 153 bp). The screening of exon 7 mutation among the GDM women using RFLP analysis demonstrated no mutation in controls or cases samples as shown in Figure 1B.

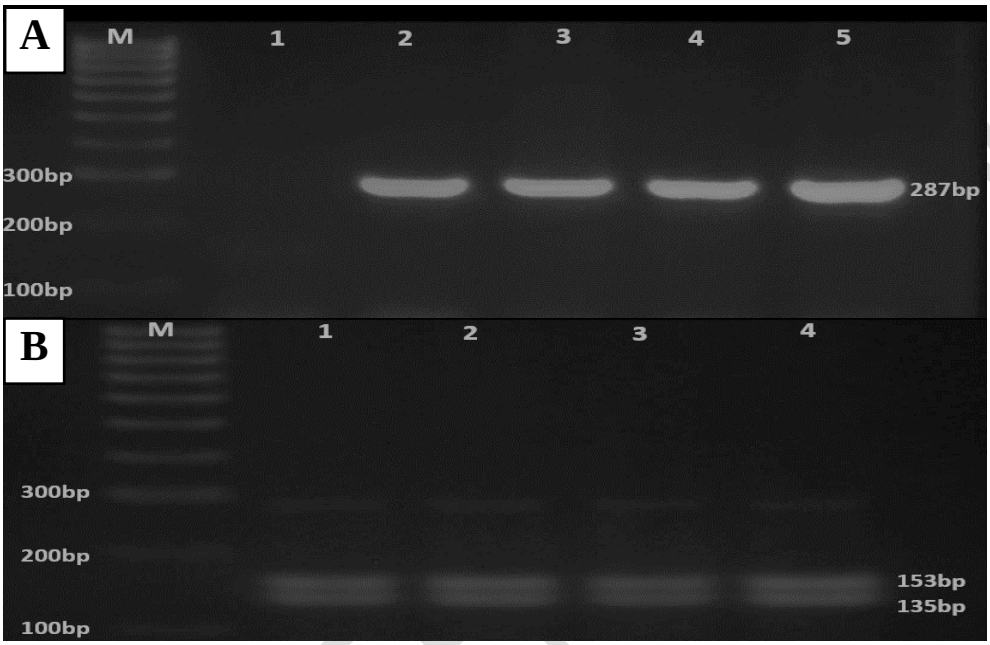

Figure (1): PCR & RFLP products of Exon 7 in *GCK* gene. (A) A photo of ethidium bromide-stained 3% agarose gel showing PCR products of Exon 7 in GCK gene. M: DNA ladder (100bp); Lane 1: Blank negative control; Lanes (2-5): Represent PCR products (287bp). (B) PCR-RFLP products of exon 7 in *GCK* gene. Restriction enzyme products were electrophoresed on 3% agarose gel containing ethidium bromide. M: DNA ladder (100bp); Lanes (1-4) illustrate samples with no mutation in GDM women, indicated by homozygous (A/A) for wild type product (153 & 135bp).

PCR and PCR-RFLP products of exon 8

Figure 2 illustrates the PCR and restriction enzyme products of exon 8 to detect the p.Gly299Arg missense mutation at nucleotide 895 (G>C). The (*HhaI*) restriction enzyme digestion for the wild-type allele yields two fragments (116 & 152 bp), while the mutant allele lacks the *HhaI* recognition site (268 bp product). The screening of exon 8 mutation among the GDM women using RFLP analysis showed that 9 of the GDM women were heterozygous for this mutation.

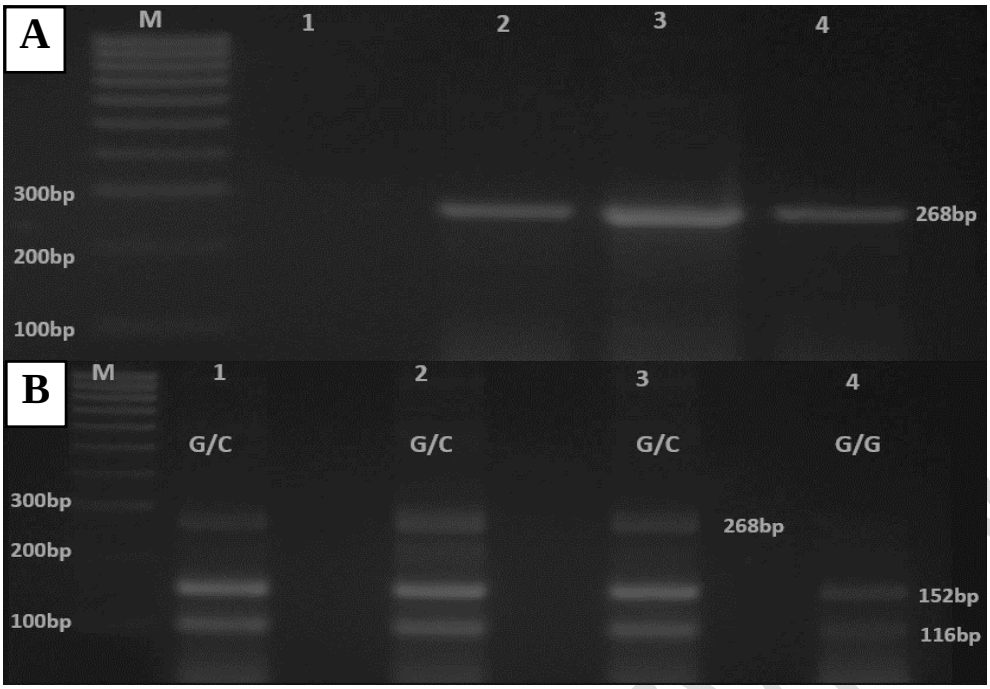

Figure 2: PCR & RFLP products of Exon 8 in *GCK* gene. **(A)** A photo of ethidium bromide-stained 3% agarose gel showing PCR products of *GCK* exon 8. M: DNA ladder (100bp); Lane 1: Blank negative control; Lanes (2-4): represent PCR products (268bp). **(B)** Restriction enzyme products were electrophoresed on 3% agarose gel containing ethidium bromide. M: DNA ladder (100bp); Lanes (1-3) illustrate heterozygous subjects, (G/C) genotype, as one allele yields (268 bp) while the other one yields two fragments (116 bp & 152 bp); Lane 4: demonstrates a wild-type (G/G) genotype (116 bp & 152 bp).

PCR and PCR-RFLP Products of Exons 9

Figure 3 shows the PCR and restriction enzyme products of exon 9 to detect the nonsense mutation (p.Ser383Ter) at nucleotide 1148 (C>A). A *BfaI* restriction enzyme recognizes two sites in the mutant allele (410 bp) and gives three fragments (323, 75 & 12 bp), while the wild-type allele has one site and produces two fragments of (398 & 12 bp). The screening of exon 9 mutation among the GDM women using RFLP analysis revealed no mutation in control or GDM women.

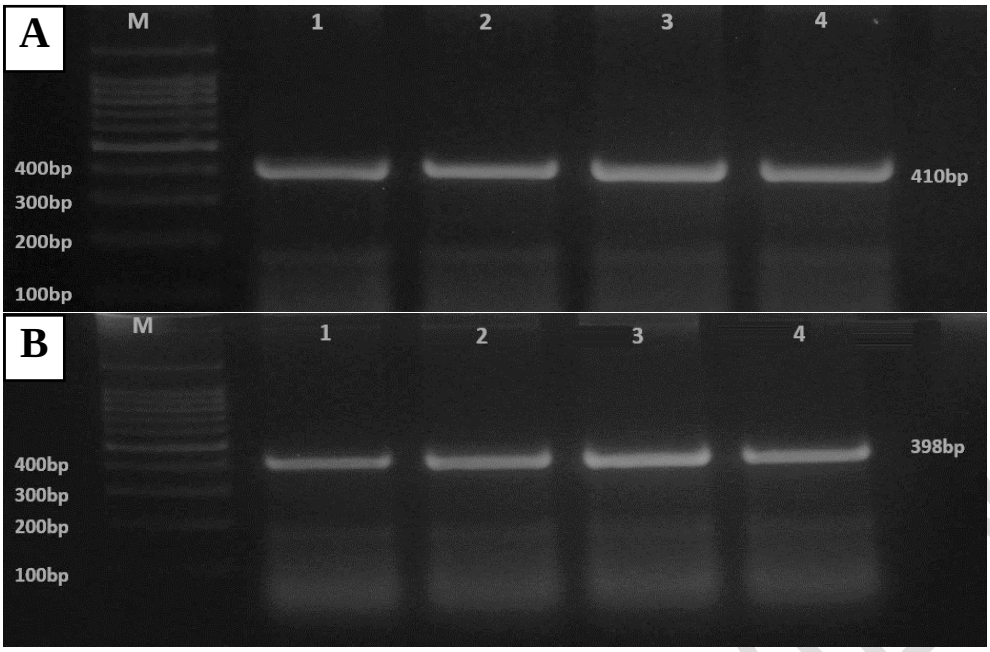

Figure 3: PCR & RFLP products of Exon 8 in *GCK* gene. **(A)** A photo of ethidium bromide-stained 3% agarose gel showing PCR of Exon 9 of *GCK* gene. M: DNA ladder (100 bp); Lane 1: Blank negative control; Lanes (2-4) represent PCR products (410 bp). **(B)** Restriction enzyme products were electrophoresed on 3% agarose gel containing ethidium bromide. M: DNA ladder (100bp); Lanes (1-4) show restriction products of *BfaI* (large band 398 bp).

Genotypes frequencies of the screened mutations in cases and controls

Table (2) illustrates the genotype frequencies of mutations [c.682A>G, p.T228A; c.895G>C, p. G299R and c.1148C>A, p. S383X)] in *GCK* exons 7, 8 and 9, respectively. In exons 7 & 9, the p.Thr228Ala and p.Ser383Ter frequency of the wild type homozygotes (AA) & (CC) were 100%, respectively. On the other hand, the frequency of p.Gly299Arg wild type genotype (GG) was 80%, and that of the heterozygous (GC) was 20%.

Table 2: *GCK* gene mutations among the study population.

Mutation	Genotype	Controls (n=42) n (%)	Cases (n=45) n (%)	P-value
p.Thr228Ala (Exon 7)	AA	42 (100.0)	45 (100.0)	1.000
	AG	0 (0.0)	0 (0.0)	
p.Gly299Arg (Exon 8)	GG	42 (100.0)	36 (80.0)	**0.002***
	GC	0 (0.0)	9 (20.0)	
p.Ser383Ter (Exon 9)	CC	42 (100.0)	45 (100.0)	1.000
	CA	0 (0.0)	0 (0.0)	

*P-value significant at P≤0.05.

General characteristics of the study population

As shown in Table 3, there is a significant statistical difference between cases and controls means in terms of weight, height, BMI, family history of DM/GDM and FBG (P<0.01).

Table 3: General characteristics of the study population.

General characteristics	Controls (n=42) Mean ± SD	Cases (n=45) Mean ± SD	P-value
Age (years) (Min-max)	29.3 ± 3 (25-35)	30.2 ± 3.3 (25-35)	0.182
Height (cm) (Min-max)	157.4 ± 6.8 (141-171)	161.3 ± 5.9 (151-175)	**0.006***
Weight (Kg) (Min-max)	70.5 ± 9.5 (58-98)	89.1 ± 10.6 (64-110)	**<0.001***
BMI (Kg/m^2) (Min-max)	28.6 ± 4.4 (22.9-41.7)	34.3 ± 4.5 (24.1-41.4)	**<0.001***
	n (%)	**n (%)**	**P-value**
Family history of DM			
Yes	11 (26.2)	26 (57.8)	**0.003***
No	31 (73.8)	19 (42.2)	
Family history of GDM			
Yes	0 (0)	10 (22.2)	**0.001***
No	42 (100)	35 (77.8)	

BMI: Body mass index; **DM:** Diabetes mellitus; **GDM:** Gestational diabetes mellitus; **n:** number of the subjects; **SD:** Standard deviation; **t:** student t-test; **χ^2:** Chi-square test. *P-value significant at P≤0.05.

Measured and calculated parameters among the study population

As presented in Table 4, there is a significant statistical difference between cases and controls regarding the means for FBG, OGTT, HbA1c, Insulin, HOMA-IR, Triglycerides, cholesterol and LDL (P<0.001).

Table 4: Measured and calculated parameters among the study population.

Parameters	Controls (n=42) Mean ± SD	Cases (n=45) Mean ± SD	P-value
FBG (mg/dl) (Min - max)	66.1 ± 8.2 (53-85)	105.8 ± 16.2 (88-175)	**< 0.001***
OGTT (mg/dl) (Min - max)	85.4 ± 8.2 (71-100)	187 ± 25.5 (145-293)	**< 0.001***
HbA1c (%) (Min - max)	4.4 ± 0.4 (3.7-5.3)	7.1 ± 0.5 (6.2-8.3)	**< 0.001***
Insulin (uIU/ml) (Min - max)	6.2 ± 1.7 (3.4-9)	20.4 ± 8.4 (6.3-41.2)	**< 0.001***
HOMA-IR (Min - max)	1.0 ± 0.3 (0.5-1.8)	5.3 ± 2.2 (1.9-12.1)	**< 0.001***
Triglycerides (Min - max)	115 ± 39.6 (42-209)	150.7 ± 40.2 (40-216)	**<0.001***
Cholesterol (Min - max)	159.6 ± 44.5 (85-273)	198.8 ± 55.2 (101-291)	**<0.001***
HDL (Min - max)	45.4 ± 7.9 (33-64)	45.6 ± 10.7 (30-69)	0.915
LDL (Min - max)	91.2 ± 43.1 (20-207)	123 ± 54.1 (25-206)	**0.003***

FBG: Fasting blood glucose; **HbA1c:** Hemoglobin A1c; **HDL: H**igh-density lipoprotein; **HOMA-IR:** Homeostatic Model Assessment for Insulin Resistance; **LDL:** low-density lipoprotein; **OGTT:** oral glucose tolerance test; **n:** number of the subjects; **SD:** standard deviation; **t:** student t-test; **TG:** Triglyceride. P-value significant at $P \leq 0.05$.

The relationship between exon 8 p.Gly299Arg mutation and the studied parameters among the study population

Table 5 shows the comparison between controls, mutation-negative cases and mutation-positive (GC) cases. Results showed that there is a statistically significant difference in terms of FBG, OGTT, HbA1c, Insulin and HOMA-IR ($P<0.001$). Moreover, there was a statistically significant difference in OGTT between mutation-negative (GG) cases and mutation-positive (GC) ones ($P=0.04$), where the concentration of glucose in mutation-positive (GC) cases was lower as compared to mutation-negative (GG) ones. The lipid profile parameters showed significant statistical difference in Triglycerides, Cholesterol & LDL ($P<0.05$). However, there was no statistically significant difference in the HDL levels.

Table (5): The relationship between p.Gly299Arg (Exon 8) mutations and the different studied parameters among the study population.

Parameter	Controls (n=42) Mean ± SD	Cases		P-value
		Mutation-negative (n=36) Mean ± SD	Mutation-positive (n=9) Mean ± SD	
BMI (Kg/m^2) (min-Max)	28.6 ± 4.4 (22.9-41.7)	34.9 ± 4.5 (24.1-41.4)	32.0 ± 3.7 (26.8-38)	0.000[a] 0.105[b] 0.211[c]
FBG (mg/dl) (Min - max)	66.1 ± 8.2 (53-85)	106.2 ± 17.1 (88-175)	104.3 ± 12.8 (89-126)	<0.001[a] <0.001[b] 0.928[c]
OGTT (mg/dl) (Min - max)	85.4 ± 8.2 (71-100)	190.6 ± 26.2 (145-293)	172.7 ± 16.5 (147-196)	<0.001[a] <0.001[b] 0.040[c]
HbA1c (%) (Min - max)	4.4 ± 0.4 (3.7-5.3)	7.1 ± 0.5 (6.2-8.3)	7.0 ± 0.4 (6.4-7.7)	<0.001[a] <0.001[b] 0.867[c]
Insulin (uIU/ml) (Min - max)	6.2 ± 1.7 (3.4-9)	20.5 ± 7 (6.3-34.5)	20.3 ± 13.2 (7.6-41.2)	<0.001[a] <0.001[b] 0.999[c]
HOMA-IR (Min - max)	1.0 ± 0.3 (0.5-1.8)	5.3 ± 1.8 (1.9-9.2)	5.1 ± 3.4 (2.1-12.1)	<0.001[a] <0.001[b] 0.975[c]
Triglycerides (Min - max)	115 ± 39.6 (42-209)	153.4 ± 40.6 (40-216)	139.9 ± 39.0 (87-205)	<0.001[a] 0.243[b] 0.664[c]
Cholesterol (Min - max)	159.6 ± 44.5 (85-273)	200.4 ± 53.5 (101-291)	192.1 ± 64.8 (102-276)	0.003[a] 0.222[b] 0.907[c]
HDL (Min - max)	45.4 ± 7.9 (33-64)	45.4 ± 11.0 (30-69)	46.6 ± 10.1 (37-67)	1.000[a] 0.351[b] 0.947[c]
LDL (Min - max)	91.2 ± 43.1 (20-207)	124.4 ± 52.6 (25-206)	117.6 ± 62.8 (40-198)	0.015[a] 0.531[b] 0.934[c]

FBG: Fasting blood glucose; **HbA1c:** Hemoglobin A1c; **HDL:** High-density lipoprotein; **HOMA-IR:** Homeostatic Model Assessment for Insulin Resistance; **LDL:** Low-density lipoprotein; **n:** Number of the subjects; **OGTT:** Oral glucose tolerance test; **SD:** Standard deviation; **TG:** Triglyceride. P-value significant at P≤0.05. **a:** compare controls (GG) versus

GDM cases (GG); **b:** compare controls versus MODY cases (GC); **c:** compare GDM cases (GG) versus MODY cases (GC).

Discussion

Knowing the genetic variants and mutations that are associated with complex genetic disorders is clinically important for identifying the genetic factors underlying those conditions. Several studies have suggested that mutations and polymorphisms in the *GCK* gene represent a pattern of MODY (Gidh-Jain et al., 1993, Stoffel et al., 1993, Velho et al., 1997, Zouali et al., 1993). The aim of this study was to identify mutations in three exons (7, 8 & 9) of *GCK* gene at positions: c.682A>G; c.895G>C and c.1148C>A in women with GDM in Gaza Strip- Palestine, and to investigate their relationship with different biochemical parameters.

Mutations in the *GCK* gene could be a common cause of gestational diabetes, at least in certain populations (Bonfig et al., 2011, Mantovani et al., 2003, Saker et al., 1996, Stoffel et al., 1992). In the current study, we could detect the exon 8 p.Gly299Arg mutation in a heterozygous form in 9 of the 45 GDM women. This finding is in agreement with those of Saker et al. (1996) who found the p.Gly299Arg mutation in 3 out of 50 UK Caucasian GDM women (Saker et al., 1996). Ellard et al. (2000) also reported a high prevalence in the *GCK* gene mutations among their GDM women, where 12 out of 15 harbored mutations (Ellard et al., 2000). In an Indian prospective study, the authors identified 13 (26%) GA genotype in cases (Swapna R and Kamineni V, 2018). However, in contrast to our results, Hassan et al. (2016) investigated this particular mutation in Saudi Arabia but did not report it in their GDM pregnant women (Hassan et al., 2016).

Exon 7 (p.Thr228Ala) c.682 (A>G) and exon 9 (p.Ser383Ter) c.1148 (C<A) *GCK* mutations have been shown to be associated with MODY-2 (Mantovani et al., 2003, Marotta et al., 2005). In the present study, the RFLP analysis of *GCK* mutations confirmed the absence of those mutations in our study population; a finding consistent with other studies (Allan et al., 1997, Hassan et al., 2016). This could be due to genetic background differences as frequency of *GCK* gene variants vary considerably among ethnic groups. However, previous literatures reported the presence of those mutation in certain Caucasian populations (Mantovani et al., 2003, Saker et al., 1996).

The findings of our study showed that there was a higher mean of BMI among cases compared to controls ($P<0.001$). It is widely reported that obesity is a risk factor for both DM and GDM. The International Diabetes Federation (IDF) in 2013 stated that, "around 10.9% of pregnant women in Europe suffered from GDM; having a high BMI as a risk factor for the development of GDM" (International Diabetes Federation, 2017). Accordingly, Chu et al. (2007) found in their meta-analysis that the risk of an obese pregnant women to develop GDM is four times higher than non-obese women (Chu et al., 2007). Consequently, the National Institute for Health and Care Excellence recommends all obese pregnant women to be screened for GDM (Women's and Health, 2015). Furthermore, the BMI of p.Gly299Arg mutation-positive GDM cases of the present study was (32.0 ± 3.7 Kg/m^2). However, there was no correlation between p.Gly299Arg mutation-positive GDM cases and controls ($P=0.105$) nor between mutation-positive and mutation-negative cases.

In women with GDM, the physiological changes in lipid profile were studied deeply during pregnancy. There is a 2-3 folds increase in basal triglycerides and cholesterol concentrations with advancing gestation. The increase is more pronounced in the GDM as compared with the normal glucose tolerant pregnant woman (Catalano, 2010a). The higher concentration of estrogen and insulin resistance (IR) are thought to be responsible for the hypertriglyceridemia of pregnancy (Butte, 2000). Indeed, our results showed that the mean levels of triglycerides, cholesterol and LDL are significantly higher in cases as compared to controls with ($P=0.001$, 0.001 and 0.003), respectively. These findings are in agreement with a Pakistani study where their results showed that the means of total cholesterol and triglycerides were

significantly higher (P<0.05) in GDM group as compared to healthy participants (Khan et al., 2013). Moreover, a systematic review published by Ryckman et al. (2015) showed that triglycerides levels for women with GDM were significantly elevated in GDM pregnant women (Ryckman et al., 2015).

However, in the present study, differences in the mean levels of HDL between cases and controls were not significant (P>0.05). Interestingly, one study concluded that pregnant women who have higher values of triglycerides and lower HDL values in first trimester of pregnancy are more likely to develop GDM (Muzurovic et al., 2018).

With regard to the difference in lipid profile between the p.Gly299Arg mutation-positive and mutation-negative GDM cases there was no statistically significant difference. Some studies stated that the carriers of *GCK* mutations usually show lower levels of fatty acids and triglycerides in circulation than the healthy population. Reduced *GCK* activity is likely to reduce glycolytic flux and production of both glycogen and malonyl-CoA. The latter is an important regulator of lipid metabolism. Thus, overall, hepatic fatty acid and triglycerides production and glucose metabolism would be decreased in the face of reduced *GCK* activity (Wędrychowicz et al., 2017). Lack of significant difference in our study may be attributed to *GCK* variants other than the three mutations investigated here.

Results of the present work showed that the cases group exhibited significantly higher 2-hour OGTT as compared to the control group (P<0.001). In the same line, Brankica et al. (2016) found a statistically significant difference between the GDM group and the control group regarding the use of OGTT for predicting large gestational age newborns (Brankica et al., 2016). In addition, it was reported that the prevalence of an abnormal OGTT was higher in women with class 2 and 3 obesity as compared to women with class 1 obesity (Farah et al., 2012). In another meta-analysis, the risk of developing GDM was estimated to be about two, four and eight times higher among overweight, obese and severely obese women, respectively as compared to normal-weight pregnant women (Chu et al., 2007).

Furthermore, the present study also showed a significant correlation between the control group and the p.Gly299Arg mutation-positive cases (P<0.001) in terms of FBG. The results also showed that the level of glucose in mutation-positive cases was lower as compared to the mutation-negative GDM cases. Consequently, Stride et al. (2002) reported this difference in fasting glucose and in response to an oral glucose load in MODY subjects. They have concluded that OGTT result reflects not only the degree of hyperglycemia but also the underlying genetic causes (Stride et al., 2002). Hence, we can see these differences between our mutation-positive cases and controls as we have just mentioned above.

Glycated hemoglobin is a widely used marker in diagnosis of DM. Consequently, some studies reported that using HbA1c can endorse diagnosis of GDM in the third gestational trimester (Capula et al., 2013, Rajput et al., 2012). In the present study, our results showed that the mean values of cases were significantly higher than controls (P<0.001). According to some studies, however, the HbA1c stays a controversial diagnostic marker during pregnancy especially in the first trimester. This is likely returned to certain conditions inherent to early stages of pregnancy, such as diversion of glucose toward the developing fetus and also to the reduced erythrocyte life span which results in lower timed exposure of new erythrocytes to glucose and thus lower glycation (Rajput et al., 2012). Moreover, the present study shows that there is a significant correlation between the control group and mutation positive cases (P<0.001). Accordingly; Gjesing et al. (2017) found this correlation when they compared the phenotypic characteristics. They reported that HbA1c was significantly higher in women with *GCK* variants compared with women without MODY gene variants (Gjesing et al., 2017).

In pregnant women, insulin hits higher levels compared with nonpregnant subjects. Consequently, IR in pregnant women with GDM appears to be greater than normal pregnant women (Catalano, 2010b, Elkind-Hirsch et al., 2010). Moreover, IR stayed higher in GDM; i.e.,

insulin sensitivity gradually declines to 50% which could be due to multiple factors such as increased levels of progesterone, estrogen, human placental lactogen and other factors.

In the current study, we observed that insulin level was significantly different between GDM women and controls (P<0.001). Sonagar et.al. (2014) found that fasting serum insulin was significantly higher in the 2nd and 3rd trimester pregnant women compared to non-pregnant women (Sonagra et al., 2014). Furthermore, Kautzky-Willer et al. (1997) have evaluated β-cell function in patients with GDM and in nondiabetic pregnant controls by estimating insulin secretion and sensitivity using minimal model calculations. They found that during late gestation, patients with GDM were more IR and secreted more insulin than nondiabetic pregnant controls (Kautzky-Willer et al., 1997). However, when the capacity of insulin secretion is not sufficiently large to meet the resistance, glucose intolerance develops and the women develop GDM (Buchanan and Xiang, 2005).

In the present study, the HOMA-IR results show that the mean values of the GDM cases were significantly higher than the controls (P<0.001). Consequently, Endo et al. (2006) investigated the changes in insulin sensitivity using HOMA and the quantitative insulin sensitivity check index (QUICKI) in normal-weight and overweight women with normal glucose tolerance (NGT) and GDM during pregnancy. They found that HOMA-IR in women with GDM increased significantly (P<0.05) during pregnancy, but HOMA-IR values in normal-weight and overweight women with NGT did not change significantly with advance of gestation. The study presented that insulin sensitivity in women with GDM declined with advance of gestation (Endo et al., 2006). Furthermore, Tanaka et al. (2018) concluded that the degree of IR at the diagnosis of GDM was a marker of GDM severity and pathophysiological heterogeneity (Tanaka et al., 2018).

Conclusion

In Conclusion, the present case-control study screened 45 GDM cases for the following *GCK* missense mutations (p.T228A, p.G299R and p.S383X). Nine of the GDM subjects harbored the p.G299R mutation with a prevalence of 20%. Detection of this mutation explains the cause of gestational diabetes in those 9 subjects. The mean levels of FBG, OGTT, HbA1c, insulin, HOMA-IR, cholesterol, triglycerides, LDL & BMI were found to be significantly higher in cases as compared to controls. Making the diagnosis of GCK-MODY through genetic testing is essential to avoid unnecessary treatment and investigations. Indeed, the results may help physicians to manage better pregnant women with GDM.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not for- profit sectors.

Ethics declarations

An approval to perform the study was taken from the Palestinian Ethical Committee (Helsinki Ethics Committee) No. PHRC/HC/240/17 and Palestinian Ministry of Health. Informed consent was taken from all women who accepted to participate in the study after well explanation of the procedures and objectives and considerations beyond the study.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of

knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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