British Journal of Medicine & Medical Research
13(8): 1-10, 2016, Article no.BJMMR.23928
ISSN: 2231-0614, NLM ID: 101570965

SCIENCEDOMAIN *international*
www.sciencedomain.org

Screening for Glucokinase (GCK) Gene Mutation in Gestational Diabetes Women in Western Region of Saudi Arabia

Sabah M. Hassan[1,2*], Archana P. Iyer[3] and Fahad A. Al-Abbasi[2]

[1]Department of Biochemistry, Al-Farabi College of Dentistry and Nursing, Jeddah, Saudi Arabia.
[2]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
[3]Department of Biochemistry and Experimental Biochemistry Unit, King Fahad Medical Research Center, Saudi Arabia.

Authors' contributions

This work was carried out in collaboration between all authors. Author API designed the study and wrote the protocol. Author SMH managed the literature searches, analyses of the study performed the Restriction Fragment Length Polymorphism analysis and wrote the first draft of the manuscript. Author API managed the experimental process and author FAAA identified the species of plant. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/23928
Editor(s):
(1) Alex Xiucheng Fan, Department of Biochemistry and Molecular Biology, University of Florida, USA.
Reviewers:
(1) Adham Ahmed, University of Palestine, Palestine.
(2) Susai Rajendran, RVS School of Engineering and Technology, Dindigul, India.
Complete Peer review History: http://sciencedomain.org/review-history/13182

Original Research Article

Received 29th December 2015
Accepted 25th January 2016
Published 6th February 2016

ABSTRACT

Aim: The study involved screening of three exons of glucokinase gene (7, 8 and 9) to detect mutations at positions 682A>G, p.T228A, 895G>C, p.G299R and 1148C>A, p.S383X, respectively in Saudi pregnant women with or without gestational diabetes.
Study Design: Retrospective study.
Place and Duration of Study: The Obstetrics and Gynecology clinic of King Abdul-Aziz Hospital in South Jeddah between May and October 2013.
Methodology: A sample of 100 Saudi pregnant women classified as healthy (control, N = 50 (pregnant women without diabetes)) or with gestational diabetes (N = 50) was analyzed for mutations in the GCK gene. Blood samples were taken and DNA was extracted from whole blood.

**Corresponding author: E-mail: sbah1430@gmail.com;*

The target part of GCK gene was amplified by the polymerase chain reaction (PCR). The specific amplified regions were digested by restriction enzymes*: Bst*UI for exon 7, *Hha*I for exon 8 and *Bfa*I for exon 9, and electrophoresed on agarose gel. Mutations were screened by restriction fragment length polymorphism.

Results: Fasting glucose levels were statistically higher in gestational diabetes women than controls (P-value = 0.00). The difference in Glucose Challenge Test (GCT) values between two groups shows significante p-value = 0.00. However, We observed that there was no significant correlation between any of the GCK mutations and gestational diabetes in Saudi population.

Conclusion: The glucokinase gene mutations do not affect glucose metabolism in pregnant Saudi women.

Keywords: Gestational diabetes mellitus; glucokinase gene; Saudi women; mutation.

ABBREVIATIONS

Bacteroides fragilis	*BfaI*
Body Mass Index	*BMI*
Bacillus stearothermophilus	*BstUI*
Ethylenediaminetetraacetic Acid	*EDTA*
Glucokinase	*GCK*
Glucose Challenge Test	*GCT*
Gestational Diabetes Mellitus	*GDM*
Haemophilus Haemolyticus	*HhaI*
Maturity-Onset Diabetes of the Young	*MODY*
Oral Glucose Tolerance Test	*OGTT*
Polymerase Chain Reaction	*PCR*
Restriction Fragment Length Polymorphism	*RFLP*
Single Strand Conformational Polymorphism	*SSCP*

1. INTRODUCTION

Diabetes mellitus is the most prevalent metabolic disorder which causes serious effects on the health of patients, and develops long-term damage, with dysfunction of various organs. It is characterized by elevated levels of blood glucose (chronic hyperglycemia). Any degree of rise of blood glucose or glucose intolerance during pregnancy is classified as gestational diabetes [1]. This condition is related with higher risk of development of diabetes mellitus type 2 after pregnancy ranging from 17%- 63% [2]. Also, it increases the risk for pregnant mothers and fetuses for gestational complications [3].

In Saudi Arabia, abnormal glycemic control affects 8.9-12.5% of pregnancies depending upon the region and also on the method of testing used [4,5]. The prevalence of gestational diabetes in Jeddah is about 12.5% of pregnant woman [6]. This value is much higher than that reported for Europeans, [7] Australians, [8] Americans, [9] and in Saudis living in Dammam [10] or Riyadh [11] areas and well-matched to Bahrain (12.5%) [12], but lower compared to Qatar (16.3%) [13] and in United Arab of Emirates (20.6%) [14].

Glucokinase (GCK) enzyme has a significant function in glucose homeostasis [15]. Glucokinase promotes insulin release from pancreatic beta cells in response to glucose sensor [16]. In chromosome 7, the human GCK gene is situated specifically in the short arm of the chromosome(7p15.3-p15.1) [17]. This gene is about 52 kb long and has 10 exons according to the reference sequence on NCBI [18].

GCK gene mutation leads to various diseases. GCK heterozygous mutations lead to Maturity-Onset Diabetes of the Young (MODY-2), a monogenic subtype of diabetes mellitus that is characterized by reduced GCK enzyme activity, whereas homozygous mutations cause a complete loss of enzyme activity [19-21].

MODY has common characteristics that also reported in GDM [22]. In fact, today it is suggested that the GCK mutations causing MODY2 could be also the cause of GDM.

Detection of GCK mutations in the gestational diabetes mellitus (GDM) population is important. Identification of the genetic causes may be useful to know the pathophysiological mechanism of the disease which helps us to reduce, treat and prevent the complications for the mother and fetus. Until now, little information about the genetic basis of GDM and the potential possibilities for treatment.

The study involved screening mutations of the three exon of GCK gene of pregnant subjects from the population of Jeddah with or without gestational diabetes to conclude if there was an association of the GCK gene with gestational diabetes in Saudi population.

2. METHODOLOGY

2.1 Subjects

The GCK gene mutation analysis study included a total of 100 pregnant Saudi women who reported to the Obstetrics and gynecology clinic of King Abdulaziz hospital. The study was approved by the ethical committee. All participants in the study filled a questionnaire and also signed a consent form. Control group consisted of 50 pregnant women with normal glucose levels while women with elevated sugar levels were classified as gestational diabetic based on the criteria defined by the American Diabetes Association [23]. Pregnant women with type I, type II diabetes or cancer were excluded from the study.

This criterion depends upon two tests: Glucose Challenge Test (GCT) and Oral Glucose Tolerance Test (OGTT). In women who are already at risk for GDM, OGTT was directly performed.

A pregnant woman was initially screened by measuring the plasma or serum glucose concentration 1 h after a 50-g oral glucose load (glucose challenge test [GCT]). If the value above 140 mg/dl (7.8 mmol/l), the OGTT was performed. After an overnight fast of between 8 and 14 h, fasting glucose value was determined (should be ≤ 95 mg/dl (5.3 mmol/l)) followed by drinking a 75-g oral glucose load. Blood glucose level was evaluated after 1h (should be ≤ 180 mg/dl (10 mmol/l)) and 2h (should be ≤ 155 mg/dl (8.6 mmol/l)). If two or more of the venous plasma concentrations exceeded the normal value, the woman was categorized as gestationally diabetic. In women who are already at risk for GDM, OGTT was directly performed.

We recorded the clinical parameters for each gestational diabetic pregnant woman upon diagnosis: age, GCT test, OGTT test, weight and height. These medical details were obtained from patients' hospital files after obtaining the consent from the administration. The previous history of GDM and family history of diabetes were taken from pregnant women. The same parameters were recorded for the control pregnant women.

2.2 Study Design

Peripheral blood samples 2-3 ml were withdrawn from the pregnant women in EDTA tubes and DNA was extracted using Qiagen DNA extraction kit. DNA was stored at -20°C for amplification. The specific regions in GCK gene (exon 7,8 and 9) were amplified by polymerase chain reaction (PCR). The primers sequence [24] (Metabion International AG) was shown in Table 1. For Polymerase Chain Reaction (PCR), the master mix from thermo scientific was used. The mixture (50 µl) contained 2X reaction buffer, 0.2 µM of each primer, 200 µM deoxyribonucleoside triphosphates, 2 mM Mg^{+2}, 10 - 30 ng of DNA template and 0.40 U Taq DNA polymerase. The volume of reaction was complete to 50 µl with nuclease free water. The PCR tubes were transferred to thermal cycle. The reaction cycle is shown in Fig. 1. The PCR products were verified by 2% agarose gel electrophoresis at 100 V for 30 min. Purification of PCR product Using PCR Clean-Up System from Promega.

2.3 Restriction Fragment Length Polymorphism (RFLP)

2.3.1 Screening of missense mutation in exon 7

To screen the missense mutation (228 T<A) in exons 7, the amplified PCR products for exons 7 was cleaved using *Bacillus stearothermophilus* (*Bst*UI) restriction endonuclease (New England BioLab). The mixture of PCR products, 5 units of restriction endonuclease and 1X CutSmart buffer that contains Bovine Serum Albumin was placed in thermal cycle that setting at 60°C that is the optimum temperature of the enzyme activity for 4-8 hours. The control that was used for check of enzyme activity was PCR product of exon 9 (it contains 3 restriction sites of *Bst*UI). Electrophoresis was performed for the digestion product on 2-3% agarose ethidium bromide stained gels at 100 V for 45 min.

2.3.2 Screening of missense mutation in exon 8

The missense mutation (G299R) in exons 8 was screened by dealing the PCR product with *Haemophilus haemolyticus* (*Hha*I) restriction endonuclease (New England BioLab). The PCR product was mixed with 5-7units of *Hha*I enzyme and 1X CutSmart buffer supplemented with enzyme. The mix was incubated overnight at 37°C, and then the enzymes were inactivated by incubation at 65°C for 20 min. Some samples were digested in the first run were used as control for each run of digestion. The digested products were verified by 2-3% agarose gel electrophoresis at 100 V for 45 min.

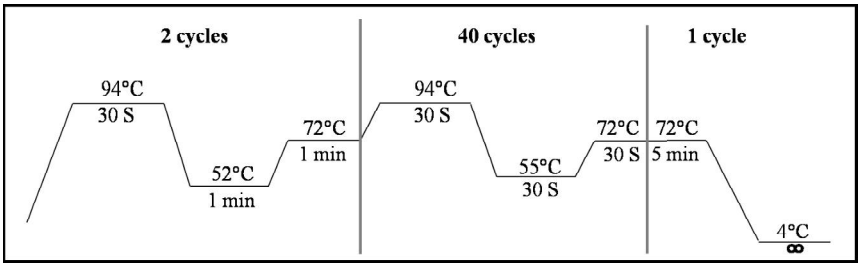

Fig. 1. Schematics of PCR condition

Table 1. The primer sequence and restriction enzymes of screened GCK gene

GCK gene		Primer sequence	Restriction enzyme
Exon7	F	5'-TGCAGCTCTCGCTGACAGTCC -3'	*Bst*UI
	R	5'- CTCCCATCTGCCGCTGCACC -3'	
Exon 8	F	5'- CGTGCCTGCTGATGTAATGG -3'	*Hha*I
	R	5'-GCCCTGAGACCAAGTCTGC -3'	
Exon 9	F	5'-CTGTCGGAGCGACACTCAG -3'	*Bfa*I
	R	5'- CCCCCAAATCTAGGCCAAGG -3'	

2.3.3 Screening of nonsense mutation in exon 9

The PCR product of exon 9 of all pregnant women was cleaved with *Bacteroides fragilis* (*Bfa*I) restriction endonuclease for screening of nonsense mutation (S383X). The mixture of PCR products, 10 units of *Bfa*I enzyme, 1X CutSmart buffer and additive Bovine Serum Albumin (1X) was incubated overnight at 37°C. Some samples were digested in the first run were used as control for each run of digestion. The digested products were verified by 12% of polyacrylamide stained gel. DNA loading dye (R0611), DNA ladder ranging from 0.05-1 kb from promega (USA) and 1X TBE buffer were used in each run. The gel was visualized under UV using Gel Documentation system (Syngene, Cambridge, UK).

2.4 Statistical Analysis

The SPSS software version 16.0 was used to analysis the data. Mean of data was compared between two groups (GDM women and normal (control) women) by independent sample T-test. The p-value that considered significant was less than 0.05.

3. RESULTS

3.1 The Main Characteristics of the Study Group

The Saudi pregnant women were classified according to GCT test or GTT test as normal (control) pregnant women, and gestational diabetic women (a total of 50 normal and 50 gestational diabetic women). The clinical parameters for two groups are shown in Table 2. Gestational diabetic women were heavier and had a broad significance (P-value = 0.075) of Body Mass Index (BMI) than the controls. Fasting glucose levels were statistically higher in GDM women than controls (P-value = 0.000). Most of GDM women were under treatment and maintained glycemic control by diet and did not show complications at diagnosis. The difference in GCT values between two groups shows significant p-value = 0.00. The 27% of the pregnant women have a history of diabetes in the first-degree relative and 8 women of controls have a sister or mother with GDM.

3.2 Analysis of the *Bst*UI Polymorphism in GCK Gene

The PCR products of exon 7 (Fig. 2a) of GCK gene were screened for detection of Thr228Ala missense mutation at nucleotide 682 (A < G) depend on the restriction transition profile of the *Bst*UI restriction enzyme (Table 3).

The *Bst*UI cut the mutant allele and yields two fragments (245 and 42 bp) while the wild-type allele is undigested. The screening of exon 7 mutation among the gestational diabetic women and normal control pregnant women using RFLP analysis showed no any mutation in both GDM women and normal control women (Fig. 2b). The PCR products were not cut and showed the

same model of restriction action in both groups. So, no mutation of Thr228Ala was detected in the study groups.

3.3 Analysis of the *Hha*I Polymorphism in GCK Gene

The missense mutation (Gly299Arg - 895G <C) in exon 8 is screened by *Hha*I restriction enzyme. The *Hha*I restriction enzyme can't digest mutant allele, while the wild-type allele gives two fragments (116 and 152 bp).

PCR products of exon 8 (Fig. 3a) of all GDM pregnant women and controls of this study were cut by *Hha*I enzyme and gave two fragments 116 and 152 bp that shown in Fig. 3b. So, there is no presence of mutation in the studied groups.

3.4 Analysis of the *Bfa*I Polymorphism in GCK Gene

The *Bfa*I restriction enzyme was used to find the nonsense mutation (Ser383Tyr) in exon 9 (1148C < A). A *Bfa*I recognizes two site in the mutant allele (410bp) and gives three fragments (323, 75, and 12 bp), while the wild-type allele has one site and produces two fragments of 398 and 12 bp. Since 12 bp is very small and we cannot see in the gel, we depend on the

Table 2. Clinical and biochemical characteristics of gestational diabetic women and controls

Parameters	Normal pregnant women	Gestational diabetic women	P-value
	Mean ± SD	Mean ± SD	
Age (years)	29±5.17	32±6.29	0.104
Weight (Kg)	69.18±14.94	81.09±27.89	0.042
BMI	28.09±5.31	32.21±5.85	0.075
Gestational weeks	29.32±6.2	29.57±5.97	0.858
GCT (mmol/L)	6.4±1.46	9.96±2.03	0.000
GTTF (FBS) (mmol/L)	4.29±0.66	5.32±0.71	0.000
GTT1 (mmol/L)	7.61±1.42	11.45±1.28	0.000
GTT2 (mmol/L)	6.57±1.22	10±1.26	0.000
Positive history of diabetes %	54%	14%	—
Negative history of diabetes %	30%	6%	—
Unknown history of diabetes %	16%	80%	—

Data is represented as mean ± SD. BMI: Body Mass Index; GCT: Glucose Challenge Test; GTTF: Glucose Tolerance Test Fasting; FBS: Fasting Blood Sugar; GTT1: Glucose Tolerance Test after 1 hour; GTT2: Glucose Tolerance Test after 2 hour

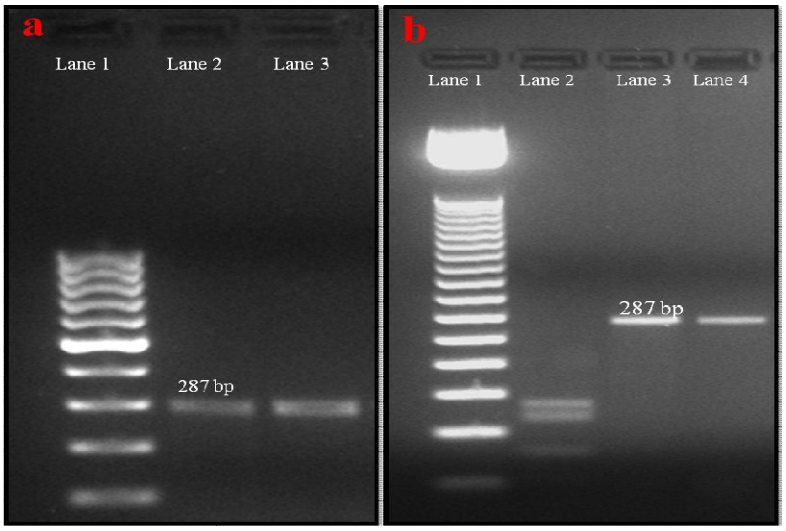

Fig. 2. a) 2% agarose gel showing PCR products of exon 7 of GCK gene. Lane 1: DNA ladder (100 bp). Lane 2-3: PCR Products (287 bp). b) 3% agarose gel showing restriction products of BstUI enzyme in exon7. Lane 1: DNA ladder (50 bp). Lane 2: Control sample for BstUI activity. Lane 3-4: Restriction products

difference of the size between PCR product (410 bp) and digested product (398 bp) in the check of electrophoresis pattern.

Among all pregnant women, controls or GDM, we did not detect any (Ser383Tyr) mutation. The PCR products of exon 9 as shown in (Fig. 4a) for all women followed same pattern of restriction action (Fig. 4b).

4. DISCUSSION

Pregnancy is considered to be a state of insulin resistance and complex though integrated hormonal and metabolic changes and hence may reveal subclinical defects and change in carbohydrate metabolism that may develop into a state of carbohydrate intolerance, or GDM. If GDM is not treated, there is an increased possibility of both maternal complications and morbidities [25]. There are some studies that reported that the GCK mutations are related with gestational diabetes mellitus. Approximately 50% of women with glucokinase mutations without a previous diagnosis of diabetes develop GDM [26]. Detection of GCK mutation in the gestational diabetes mellitus (GDM) population is important especially in woman with diabetes in pregnancy because they have an expected medical course and the autosomal dominant inheritance means that the possibility of infecting up to 50% for each child [27].

From the previous studies, it is evident that mutations in the GCK gene seem to vary with different populations in their association with gestational diabetes. Our study therefore aimed to screen three exons for GCK mutations and to establish a possible association with gestational diabetes in the Saudi population.

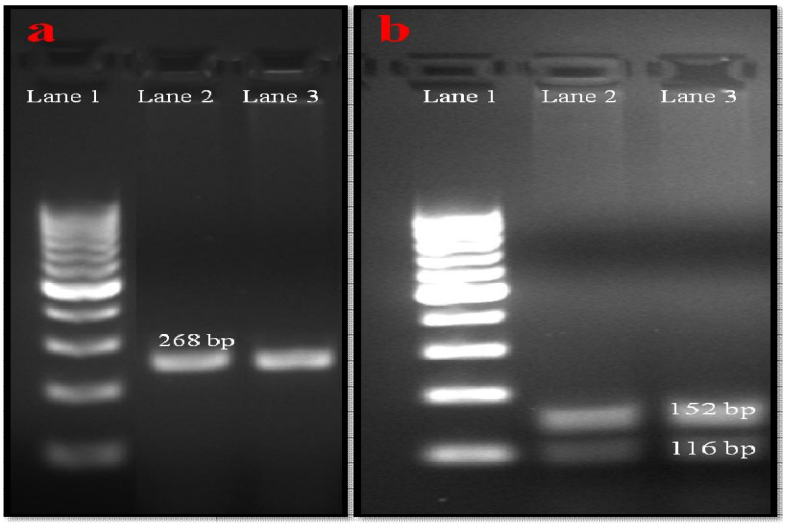

Fig. 3. a) 2% agarose gel showing PCR products of exon 8 of GCK gene. Lane 1: 100 bp DNA ladder. Lane 2-3: PCR Products (268 bp). b) 2% agarose gel showing restriction products of exon 8 of GCK gene by Hhal. Lane 1: 100 bp DNA ladder. Lane 2-3: Restriction products

Table 3. The restriction site of each enzyme and RFLPs of GCK gene

Analyzed GCK region	Restriction enzyme	Restriction site	PCR product size (bp)	RFLP Fragment size (bp)	
				Normal	Mutant
EXON 7	*Bst*UI	5' …CGᵛCG…3' 3'…..GCᐱGC…5'	287	(287) No cutting	245 42
EXON 8	*Hha*I	5' …GCGᵛC…3' 3'…..CᐱGCG…5'	268	116 bp 152 bp	No cutting
EXON 9	*Bfa*I	5' …C ᵛTAG… 3' 3'…..GATᐱC … 5'	410	398 12	323 75 12

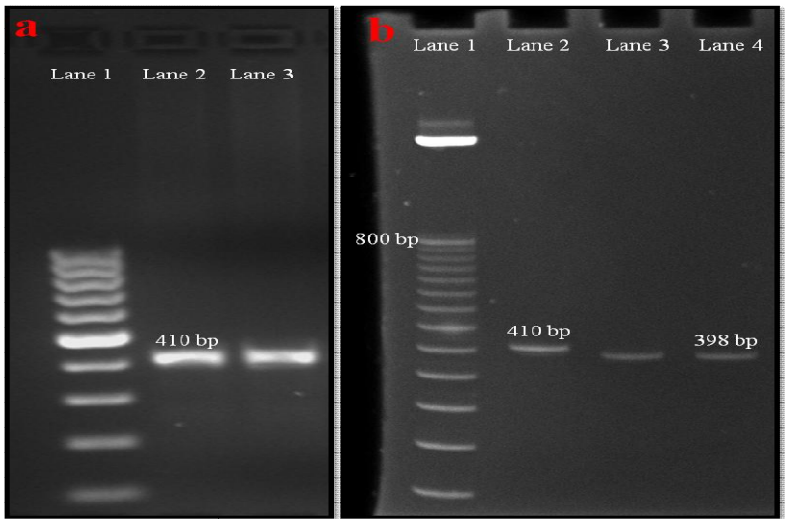

Fig. 4. a) 2% agarose gel showing PCR products of exon 9 of GCK gene. b) 12% polyacrylamide gel showing restriction products of exon 9 of GCK gene by *Bfa*I Lane 1: DNA ladder (50 bp). Lane 2: PCR product of exon 9 (410 bp). Lane 3-4: restriction products of B*fa*I (larg band 398 bp)

In the present study, the results of analysis of GCK mutations by RFLP in exon 7, 8 and 9 between two groups of pregnant women, shows no difference between gestational diabetic women and healthy controls. We did not detect any *GCK* gene mutation at positions 682A>G, 895G>C, and 1148C>A respectively, in 100 pregnant women. All pregnant women with GDM have the same electrophoresis pattern as the control group of pregnant women. So, there is no any positive cases for the GCK mutations.

Our results are in agreement with those reported by Chiu et al. [28] in 171 American Black gestational diabetic women that found no significant GCK mutations.

In addition, no mutations have been detected in any exon of the glucokinase gene by single strand conformational polymorphism analysis (SSCP) and direct exon sequencing in 50 American gestational diabetic subjects. Allan et al. [29] reported that no affect of the GCK gene mutation in the pathogenesis of GDM.

Also Zaidi et al. [30] studied 35 British Caucasian women with GDM, and used SSCP method and sequencing to detect any change in amino acids sequence and also they specifically tested the missense mutation in exon 8 by RFLP and digested the products with HhaI enzyme. They did not find any glucokinase gene mutations and Gly299Arg missense mutation as we found. This contrasts with study carried out by Saker et al. [31] who found Gly299Arg mutation in 3 out of 50 UK white Caucasian GDM women from Oxfordshire. They selected only the GDM women whose plasma glucose did not return to normal value after pregnancy and thus were not consider GDM.

Furthermore, Lucasova et al. [32] found ineffective variants and polymorphisms in exons 6 and 7 which not known as GCK pathogenic mutation when studied 141 Czech gestational diabetic women.

In contrast to our study, two mutations of glucokinase gene were reported in 40 American GDM women. The two women have a first-degree relative with diabetes. One of the these women had increased in levels of fasting blood glucose (maximum value was 130 mg/dl) from age 16 and the other women reported borderline elevated blood glucose levels at 26 yr of age and this mutations are likely to be the cause of gestational diabetes [33].

Frigeri et al. [34] detected GCK mutations in 30 of 200 gestational diabetes pregnant Brazilian women using single-strand conformation polymorphism and DNA sequencing. All mutations were in intron 3, 6, 9 and exon 10. None of these *GCK* mutations were affect glucose regulation and were not associated with GDM in a pregnant Brazilian population.

Ellard et al. [35] studied the affect of the stringent selection of gestational diabetic patients on

frequency of glucokinase mutations. This study investigated GCK pathogenic mutations in GDM in a cohort of UK Caucasians who met one of the these criteria: 1) permanence fasting hyperglycemia after pregnancy (5,5-8 mmol/l), 2) maintain glycemic control by diet but treatment with insulin at least in one pregnancy, 3) during or post pregnancy, an increase in plasma glucose level of less than 4,6 mmol/l between the fasting and 2-h in at least one 75-g oral glucose tolerance test 4) a history in first-degree relative family with gestational diabetes, type II diabetes, or fasting hyperglycemia (>5,5 mmol/l). By using these previously characteristics, Ellard et al. [35] were detected 80% of glucokinase gene mutations in gestational diabetic women. This is the greatest percentage recorded in literature.

Kousta and colleagues used the same criteria that used by Ellard et al. [36] and found 12% of GCK mutations.

In recent study, Rudland et al. [37] gave new pregnancy-specific screening criteria (NSC) to differentiate (GCK–maturity-onset diabetes of the young [MODY]) from gestational diabetes mellitus (GDM) and to specify pregnant woman who will be subject to genetic testing. The NSC criteria depend on measurement of fasting blood sugar during pregnancy that must be above 5.5 mmol/L while prepregnancy body mass index (BMI) must less than 25 mg/kg2. From 776 pregnant women, they identify 63 gestational diabetic women. Just 31 GDM women from 63 agreed to undergo GCK testing. They found 4 out of 31 GDM women were have GCK mutations.

The frequency of *GCK* gene in the three previous study was differing than we found in this study. This is due to difference in the method of selecting GDM patients.

In summary, missense or nonsense mutations in the glucokinase gene may not affect directly on the regulation of blood sugar and may be not linked significantly with gestational diabetes mellitus in Saudi population. However, our study that screened 3 exons (7, 8 & 9) did not give evidence of risk of GCK gene in the pathogenesis of gestational diabetes.

5. CONCLUSION

The present results showed that there is no relationship between GCK gene mutation and gestational diabetes women in Saudi Arabia. Mutations in glucokinase gene do not form any genetic risk for gestational diabetes. So, no major function of glucokinase gene in the causing of GDM.

ETHICAL APPROVAL

The study was approved by Scientific Committee and the Ethics Committee of Scientific Research that are registered at the National Commission for bio-medical ethics with number (H-02-J-002), Jeddah – Saudi Arabia: date of approval 29 April 2013, reference number:00137.

ACKNOWLEDGEMENTS

I wish to thank Experimental Biochemistry Unit and lab center in King Fahd Medical Research Center for equipments facilities. I thank King Abdulaziz City for Science and Technology for financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes. Diabetes Care. 2012;35:11-63.
2. Lobner K, Knopff A, Baumgarten A, Mollenhauer U, Marienfeld S, Garrido-Franco M, Bonifacio E, Ziegler A. Predictors of postpartum diabetes in women with gestational diabetes mellitus. Diabetes Care. 2006;55:792-7.
3. Riskin-Mashiah S, Younes G, Damti A, Auslender R. First-trimester fasting hyperglycemia and adverse pregnancy outcomes. Diabetes Care. 2009;32:1639-43.
4. Al-Hakeem MM. Pregnancy outcome of gestational diabetic mothers: Experience in a tertiary center. J Fam Community Med. 2006;13:55.
5. Al-Rowaily MA, Abolfotouh MA. Predictors of gestational diabetes mellitus in a high-parity community in Saudi Arabia. Eastern Med Health J. 2010;16:636-41.
6. Ardawi MS, Nasrat HA, Jamal HS, Al-Sagaaf HM, Mustafa BE. Screening for gestational diabetes mellitus in pregnant females. Saudi Med J. 2000;21:155-60.

7. Carlotti N, Maugendre D, Laurent MC, Grall JY. Mass screening of gestational diabetes using O'Sullivan's test in a hospital consultation. Experience in Rennes. J Gynecol Obstet Biol Reprod. 1996;25:168.
8. Moses RG, Griffiths RD, Mcpherson S. The incidence of gestational diabetes mellitus in the Illawarra area of New South Wales. Aust NZ J Obstet Gyn. 1994;34: 425-7.
9. Magee MS, Walden CE, Benedetti TJ, Knopp RH. Influence of diagnostic criteria on the incidence of gestational diabetes and perinatal morbidity. Obstet Gynecol Surv. 1993;48:446-7.
10. Khwaja SS, Al Suleiman SA, Al Sibai MH. Screening for gestational diabetes in a teaching hospital in Saudi Arabia. Aust NZ J Obstet Gyn. 1989;29:209-11.
11. Al Shawaf T, Ariel A, Mogiiraby SAS. Gestational diabetes and impaired glucose tolerance of pregnancy in Riyadh. BJOG. 1988;95:84-90.
12. Al Mahroos S, Nagalla DS, Yousif W, Sanad H. A population-based screening for gestational diabetes mellitus in non-diabetic women in Bahrain. Ann Saudi Med. 2005;25:129-33.
13. Bener A, Saleh NM, Al-Hamaq A. Prevalence of gestational diabetes and associated maternal and neonatal complications in a fast-developing community: Global comparisons, Int J Womens Health. 2011;3:367-73.
14. Agarwal MM, Dhatt GS, Zayed R, Bali N. Gestational diabetes: Relevance of diagnostic criteria and preventive strategies for Type 2 diabetes mellitus. Arch Gynecol Obstet. 2007;276:237-43.
15. Matschinsky FM, Ellerman JE. Metabolism of glucose in the islets of Langerhans. J Biol Chem. 1968;243:2730-6.
16. Matschinsky FM. Assessing the potential of glucokinase activators in diabetes therapy. Nat Rev Drug Discov. 2009;8:399-416.
17. Tinto N, Zagari A, Capuano M, De Simone A, Capobianco V, Daniele G, Giugliano M, Spadaro R, Franzese A, Sacchetti L. Glucokinase gene mutations: Structural and genotype-phenotype analyses in MODY children from South Italy. PLoS One. 2008;3:e1870.
18. Ng_008847.1- National Center for Biotechnology Information. Available:http://www.ncbi.nlm.nih.gov/
19. Gloyn AL. Glucokinase (GCK) mutations in hyper- and hypoglycemia: Maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemia of infancy. Hum Mutat. 2003;22:353-62.
20. Greeley SA, Tucker SE, Worrell HI, Skowron KB, Bell GI, Philipson LH. Update in neonatal diabetes. Curr Opin Endocrin Diabetes Obes. 2010;17:13-9.
21. Rubio-Cabezas O, Klupa T, Malecki MT. Permanent neonatal diabetes mellitus - The importance of diabetes differential diagnosis in neonates and infants. Eur J Clin Invest. 2011;41:323-33.
22. Page RCL, Levy JC, Barrow B, Turner RC, Patel P, Hattersley AT, Lo D, Wainscoat JS, Permutt MA, Bell GI. Clinical characteristics of subjects with a missense mutation in glucokinase. Diabetic Med. 1995;12:209-17.
23. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2007;36:S67-S74.
24. Matschinsky FM. Regulation of pancreatic B-Cell glucokinase from basics to therapeutics. Diabetes. 2002;51:S394-S404.
25. Reed BD. Gestational diabetes mellitus. Prim Care. 1988;15:371-87.
26. Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. New Engl J Med. 2001;345:971-80.
27. Had Den DR. (Ed.) In horizons in medicine. 1 ed. Bristol, Blackwell Science. 1996;440-9.
28. Chiu KC, Go RCP, Aoki M, Riggs AC, Tanizawa Y, Acton RT, Bell DSH, Goldenberg RL, Roseman JM, Permutt MA. Glucokinase gene in gestational diabetes mellitus: Population association study and molecular scanning. Diabetologia. 1994;37:104-10.
29. Allan CJ, Argyropoulos G, Bowker M, Zhu J, Lin P-M, Stiver K, Golichowski A, Garvey WT. Gestational diabetes mellitus and gene mutations which affect insulin secretion. Diabetes Res Clin Pr. 1997;36: 135-41.
30. Zaidi FK, Wareham NJ, Mccarthy MI, Holdstock J, Kalloo-Hosein H, Krook A, Swinn RA, Rahilly S. Homozygosity for a common polymorphism in the islet specific promoter of the glucokinase gene is

associated with a reduced early insulin response to oral glucose in pregnant women. Diabetic Med. 1997;14:228-34.
31. Saker PJ, Hattersley AT, Barrow B, Hammersley MS, Mclellan JA, Lo YM, Olds RJ, Gillmer MD, Holman RR, Turner RC. High prevalence of a missense mutation of the glucokinase gene in gestational diabetic patients due to a founder-effect in a local population. Diabetologia. 1996;39: 1325-8.
32. Lukasova P, Vcelak J, Vankova M, Vejrazkova D, Andelova K, Bendlova B. Screening of mutations and polymorphisms in the glucokinase gene in Czech diabetic and healthy control populations. Physiol Res. 2008;57:99-108.
33. Stoffel M, Bell KL, Blackburn CL, Powell KL, Seo TS, Takeda J, Vionnet N, Xiang K-S, Gidh-Jain M, Pilkis SJ. Identification of glucokinase mutations in subjects with gestational diabetes mellitus. Diabetes. 1993;42:937-40.
34. Frigeri HR, Santos IC, Rea RR, Almeida ACR, Fadel-Picheth CMT, Pedrosa FO, Souza EM, Rego FGM, Picheth G. Low prevalence of glucokinase gene mutations in gestational diabetic patients with good glycemic control. Genet Mol Res. 2012;11: 1433-41.
35. Ellard S, Beards F, Allen LIS, Shepherd, M, Ballantyne E, Harvey R, Hattersley AT. A high prevalence of glucokinase mutations in gestational diabetic subjects selected by clinical criteria. Diabetologia. 2000;43:250-3.
36. Kousta E, Ellard S, Allen LIS, Saker PJ, Huxtable SJ, Hattersley AT, Mccarthy MI. Glucokinase mutations in a phenotypically selected multiethnic group of women with a history of gestational diabetes. Diabetic Med. 2001;18:683-4.
37. Rudland VL, Hinchcliffe M, Pinner J, Cole S, Mercorella B, Molyneaux L, Constantino M, Yue DK, Ross GP, Wong J. Identifying glucokinase monogenic diabetes in a multiethnic gestational diabetes mellitus cohort: new pregnancy screening criteria and utility of HbA1c. Diabetes Care. 2015;39:50-2.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13182