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

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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.

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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.
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 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 women [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(7p15.3-7p15.1) [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 Mg²⁺, 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 (BstUI) 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 BstUI). 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 (Hhal) restriction endonuclease (New England BioLab). The PCR product was mixed with 5-7 units of Hhal 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.
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* (*BfaI*) restriction endonuclease for screening of nonsense mutation (S383X). The mixture of PCR products, 10 units of *BfaI* 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 *BstUI* 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 *BstUI* restriction enzyme (Table 3).

The *BstUI* 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 HhaI Polymorphism in GCK Gene

The missense mutation (Gly299Arg - 895G <C) in exon 8 is screened by HhaI restriction enzyme. The HhaI 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 HhaI 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 BfaI Polymorphism in GCK Gene

The BfaI restriction enzyme was used to find the nonsense mutation (Ser383Tyr) in exon 9 (1148C < A). A BfaI 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 |
|---------------------------------|-----------------------|----------------------------|---------|
| 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 HhaI. 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) |
|---------------------|--------------------|------------------|-----------------------|------------------------|
| EXON 7              | BstUI              | 5' …CGvCG…3'    | 287                   | (287) 245             |
|                     |                    | 3'…GCA^GC…5'    |                       | No cutting 42          |
| EXON 8              | Hhal               | 5' …GCGvC…3'    | 268                   | 116 bp No             |
|                     |                    | 3'…GAGCG…5'     |                       | 152 bp cutting        |
| EXON 9              | BfaI               | 5' …C vTAG…3'   | 410                   | 398 323               |
|                     |                    | 3'….GATAC … 5'  |                       | 12 75 12              |
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 Hhal 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 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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