Original Article

Relationship between *PTEN* and gestational diabetes in Asian Indians womens

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ABSTRACT

Gestational diabetes mellitus (GDM) is defined as glucose intolerance of variable severity with onset of first recognition during pregnancy. GDM occurs in at least 30% of women with a family history of T2DM/GDM, suggesting that some women are genetically predisposed to develop GDM. Phosphatase and tensin homolog (*PTEN*) on chromosome 10 is a tumor-suppressor gene. Studies have demonstrated that PTEN dysfunction affects the function of insulin. However, the relationship between GDM and *PTEN* has not been studied. The aim of the present study was to investigate the relationship between *PTEN* and GDM in Asian Indian women. The case-control study used PCR-RFLP analysis to assess the *PTEN*−9C/G polymorphism in 150 GDM cases and 150 controls (non-GDM). No alleles or genotypes were detected at statistically significant frequencies. All subjects were normal, and no variants were detected in any of the pregnant women. In conclusion, *PTEN* has no role in GDM, consistent with previous studies.

Keywords: 9C/G, Asian Indians, gestational diabetes mellitus, phosphatase and tensin homolog

INTRODUCTION

Gestational diabetes mellitus (GDM) is a disease state in which underlying defects in maternal insulin sensitivity and secretion are unmasked by the metabolic stressors of pregnancy.[1] GDM is a growing health concern characterized by carbohydrate intolerance of variable severity. It usually presents during the latter half of pregnancy. Risk factors for GDM include obesity, advanced maternal age, a family history of type 2 diabetes mellitus (T2DM), a past history of GDM, previous adverse pregnancy outcomes, and high-risk ethnicity.[2] Increases in maternal age and the rate of obesity have led to a growing number of GDM cases.[3] GDM is associated with an increased rate of obstetric and neonatal complications such as preeclampsia, neonatal hypoglycemia, caesarean section and macrosomia.[3] It is thought that hormones produced during pregnancy reduce a women's receptivity to insulin, leading to high blood sugar levels in almost 10 - 16% of pregnancies.[4,5] This is important because GDM is classified as a prediabetic condition with increased maternal and perinatal risk to develop T2DM.[6,7] Currently, selective screening strategies for GDM are based on maternal and obstetric history, with recent guidelines advising to screen the subjects based on the risk factor exposures. However, there is no consensus on how best to identify patients with GDM.[8]

Phosphatase and tensin homolog 10 (*PTEN*) (also known as mutated in multiple advanced cancers 1 or transforming growth factor-β-regulated and epithelial cell-enriched phosphatase 1) is a tumour suppressor gene located on chromosome 10q23.3.[9] *PTEN* contains nine exons and encodes a 403-amino acid protein, which contains a PIP2 binding site, a phosphatase domain, a C2 domain with phosphorylation sites, and a PDZ binding motif (PSD-95, Discs-large, ZO-1) from the N-terminal to C-terminal regions.[10] *PTEN* is associated with glucose and lipid metabolism, the association of its single nucleotide polymorphisms with metabolic diseases. Four *PTEN* polymorphisms have been identified in Caucasian patients with T2DM, but they

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were not associated with the disease. Three different PTEN variants have been identified in Japanese diabetic patients, and the substitution of C with G at position −9 (−9C → G) in the 5′-untranslated region of exon 1 is associated with T2DM.[11] The present study was taken up to investigate the association of *PTEN* (−9C/G) gene with gestational diabetes women in Asian Indians. Pregnant women from different districts of Telangana and Andhra Pradesh visited the Kamineni and Muslim maternity hospitals in Hyderabad. The disease pattern in the population of pregnant women included in this study is representative of the disease pattern in these regions.

MATERIALS AND METHODS

Pregnant women

Three hundred pregnant women comprising of 150 GDM, and 150 non-GDM (control) were enrolled in this study. The study was approved by the ethical committee of the study hospitals. Qualified physicians screened and managed diabetes during pregnancy in accordance with the American Diabetes Association (ADA) guidelines. The inclusion and exclusion criteria are described in an earlier publications.[12-15]

Assortment of gestational diabetes mellitus women

To identify women with GDM, a glucose challenge test (GCT) was administered at the 24th week of gestation. Glucose (50 g) was given after >12 hours of fasting; if the plasma glucose value exceeded 130 mg/dL after 1 h, a standard oral glucose tolerance test (OGTT) protocol was used for the pregnant (or gravid) women. After an overnight fast of 12h, venous plasma samples were collected fasting, and 100-g glucose. The diagnosis of GDM was based on the criteria of the ADA. The OGTT was performed routinely between 24 and 28 weeks of gestation and occasionally at other stages of gestation if clinically warranted.[12-15]

Clinical and biochemical measurements

Clinical and anthropometric parameters, including body mass index (BMI), were calculated according to Quetelet's equation by using the weight in kilograms divided by the height in square meters (kg/m^2). Three milliliter of the plain serum sample was used to measure the glucose levels of fasting, postprandial, GCT and OGTT in GDM and non-GDM subjects.

DNA and polymerase chain reaction-restriction fragment length polymorphism exploration

Genomic DNA was isolated from the peripheral blood of the pregnant women (n = 300), collected in EDTA tube for the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The DNA samples were stored at −80°C. The genotyping was performed at Department of Genetics and Molecular Medicine (NABL accreditation laboratory), Kamineni Hospitals, Telangana, Hyderabad, India for the rs11202592 polymorphism in the exon 1 region of *PTEN* gene by PCR-RFLP, followed by agarose gel electrophoresis, using a pair of oligonucleotide primers: (Forward sequence) 5′-CTTCTGCCATCTCTCTCCTC-′3 and (reverse sequence) 5′-ACTACGGACATTTTCGCATC-′3 were used for PCR amplification. The primers were synthesized by Bioserve Biotechnology (Hyderabad, India) for PCR analysis. A three-step PCR was performed using Applied Biosystem thermal cycler (Invitrogen Foster city, United States). DNA was denatured at 95°C for 5 min, then amplified by 35 PCR cycles (95°C for 30 s, 60°C for 30 s, 72°C for 45 s) followed by final extension step at 72°C for 5 min. The reaction volume contained 100 pmol of each primer, 17.75 µL of sterile water, 6.25 µL of master mix (included $MgCl_2$, 10x Taq buffer, 10 mM dNTPs and 10 unit of Taq DNA polymerase Bangalore Genie, India) and 1 µl template DNA. The amplified 212 bp PCR product was digested with AvaII restriction enzyme (Fermentas, fast digest) by incubating at 37°C for 5 min, followed by separation of the fragments on 2% agarose gel, [Figure 1].

Statistical analysis

Data were represented as mean ± standard deviation. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) for Windows, version 19.0 (SPSS, Chicago, IL, USA). Chi-square test was used for comparison of expected and observed frequencies of categorical variables. Relative risk was calculated for the genotypes that showed a significant association with the disease. Values of P (two-tailed) < 0.05 were considered as statistically significant.

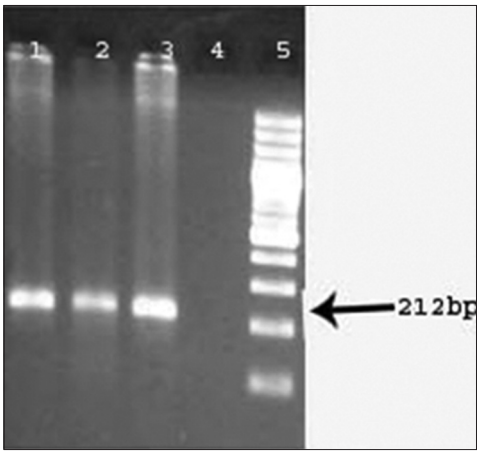

Figure 1: Electrophoresis on 2% agarose gel picture of *PTEN* gene. Lane 1: Polymerase chain reaction (PCR) product of 212 bp. Lane 2: Undigested PCR product CC genotype. Lane 3: Digested PCR product of CC genotype. Lane 4: No product. Lane 5: 100 bp DNA marker

RESULTS AND DISCUSSION

The study included 150 women with GDM and 150 women without GDM. All pregnant women were from regions of South India (Telangana and Andhra Pradesh). The clinical characteristics of women with and without GDM are shown in Table 1. Of the GDM cases, 60% had a family history. Women with GDM had significantly higher levels of fasting blood glucose and postprandial blood glucose when GDM cases and controls were compared ($P < 0.001$). The genotypic distribution of *PTEN*−9C/G and the frequency of the C and G alleles in subjects with and without GDM are shown in Table 2. There was no statistically significant difference between cases and controls in the allele frequencies or in the genotypic distribution.

This case-control study was carried out in Asian Indian women who developed diabetes during pregnancy. *PTEN* mutations in women with GDM ($n = 150$) were assessed with PCR-RFLP analysis. To our knowledge, this is the first study to assess the relationship between *PTEN* and GDM. The study demonstrated that the *PTEN* (-9C/G) polymorphism was not associated with GDM. A previous Japanese study with a different study design (case-control study) and smaller sample size (107 patients with T2DM and 100 control subjects) concluded that the *PTEN*−9C/G polymorphism was associated with T2DM because the allele frequency and the odds ratio for the frequency of the mutant genotype were significantly higher in the diabetic group than in the control group.[16] A study of the same polymorphism in a Chinese population with metabolic syndrome (530 cases and 202 controls) failed to find an association.[9] Our study is in agreement with the results of the latter study. In our population, T2DM was not associated with the *PTEN* −9C/G polymorphism (unpublished data).

Table 1: Clinical details of GDM and non-GDM women contributed in this study

Profiles	GDM ($n = 150$)	Non-GDM ($n = 150$)	*P*
Age	22 – 38 (28.8 ± 4.08)	17 – 34 (27.6 ± 3.95)	0.6
Weight	69.8 ± 10.11	66.2 ± 9.26	0.28
BMI	26.7 ± 3.64	26.1± 3.55	0.76
Mean gestational age	24.5 ± 4.90	NA	NA
FBS	109.5 ± 9.67	95.1 ± 7.67	0.004
PPBG	158.1 ± 70.71	117 ± 40.87	0.0001
Family history (%)	90 (60)	68 (45.3)	0.002
Insulin/diet (%)	57 (38)/93 (62)	NA	NA

NA: Not analyzed/not applicable, GDM: Gestational diabetes mellitus, BMI: Body mass index, FBS: Fasting blood glucose, PPBG: Postprandial blood glucose

Table 2: Genotype and allele frequency of *PTEN* gene polymorphisms in GDM

Genotype	GDM ($n = 150$) (%)	Non-GDM ($n = 150$) (%)
CC	150 (100)	150 (100)
CG	0 (0)	0 (0)
GG	0 (0)	0 (0)
C	300 (100)	300 (100)
G	0 (0)	0 (0)

GDM: Gestational diabetes mellitus

The *PTEN*−9C/G polymorphism might increase PTEN expression. PTEN antagonizes PI3K, inhibits the PI3K/PI(3,5)P3/Akt pathway, and decreases insulin sensitivity. The PI3K/PI(3,5)P3/Akt pathway is crucial for the action of insulin in regulating feeding and energy homeostasis in the hypothalamus.[17] Inhibition of the PI3-kinase pathway as a result of enhanced PTEN lipid phosphatase activity results in increased food intake and a positive energy balance, leading to obesity in rats.[18] Further studies investigating the mechanism of correlation between GDM and the *PTEN*-9C/G polymorphism are necessary to fully elucidate this.

In this study, only the G allele and GG genotype were detected; the CG and GG genotypes were not detected in GDM cases or non-GDM subjects. Because mutations in *PTEN* were not detected, we could not compare genotypes with the clinical characteristics. Pregnancy BMI is a risk factor for GDM in women from Korea and Saudi Arabia.[19-20] Pregnancy is characterized by progressive insulin resistance. GDM develops in only a small proportion of pregnant women. The insulin resistance that develops during pregnancy might result from a combination of increased maternal adiposity and the insulin-desensitizing effects of placental products such as human placental lactogen, estrogen, and prolactin. Normally, an increase in insulin secretion by pancreatic islet β-cells compensates for the increase in insulin resistance during pregnancy. Consequently, the changes in circulating glucose levels throughout pregnancy are quite small, relative to the large changes in insulin sensitivity. GDM could develop when increased insulin resistance during pregnancy unmasks a genetic predisposition towards pancreatic β-cell impairment.[21]

CONCLUSION

Our aim was to identify a significant association with *PTEN* and GDM. From the results, we conclude that *PTEN* has no role in the pathogenesis and progression of the disease and that *PTEN* was not associated with GDM in Asian Indian population. To assess the PTEN -9C/G polymorphism in different ethnicities, additional studies with a large sample size are recommended.

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