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GLUCOKINASE GENE POLYMORPHISM – RISK FACTOR IN DEVELOPING GESTATIONAL DIABETES MELLITUS,

Gynaecology

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ABSTRACT

Gestational diabetes is a common metabolic disease of pregnancy that shares many features of type 2 diabetes mellitus. Glucokinase gene encodes for human glucokinase enzyme, a major component of glucose sensing enzyme. The prevalence of glucokinase gene mutation in gestational diabetes is 0 to 6 %.

This is a prospective study conducted at Kamineni Hospital LB nagar, Hyderabad. 50 pregnant women having gestational diabetes are taken as cases and 50 pregnant women with normal sugar levels taken as controls in this study. The period of study was from April 2015 to April 2017.

We have studied hepatic GCK polymorphism in 50 cases and 50 controls. The study was done in Indian women. Their genotypes were determined by using PCR-RELP assay.

This study indicates glucokinase gene polymorphism is definitely a risk factor in developing gestational diabetes

KEYWORDS:

Gestational diabetes, Glucokinase.

INTRODUCTION :

Gestational diabetes is defined as a glucose intolerance of various degrees that is first detected during pregnancy. This definition applies whether or not insulin is used for treatment. Gestational diabetes is a common metabolic disease of pregnancy that shares many features of type 2 diabetes mellitus [1, 2, 3, 4].

The incidence of gestational diabetes is 2 to 4%. The population of India has an increased susceptibility to diabetes. This propensity was demonstrated by multiple surveys of migrant Indians residing in Fiji, Singapore, South Africa, UK and USA. The rates of diabetes in migrants from subcontinent have consistently shown to exceed those of local population [9].

Nearly 50% of women with gestational diabetes will develop type II over a period of 5-20 years. Despite more than 30 years of research there is no consensus regarding optimal approach to screening for gestational diabetes. The major issues include whether universal or selective screening should be used. Screening for gestational diabetes has failed to gain support from several national organizations. Selective screening is required for all high risk women. Genetic studies will help in identifying women at high risk of developing gestational diabetes.

The genetic basis of diabetes is undisputed. Twin studies showed that identical twins that develop diabetes have a 90% concordance thus showing a strong genetic component. Genetic studies may help in identifying women whose beta cells will tolerate insulin resistance poorly as well as women who develop poor secretion of insulin.

Better understanding of mechanisms that can lead to gestational diabetes should allow more rational development and administration of therapy during pregnancy as well as more rational approach to prevent both gestational diabetes and diabetes after pregnancy.

Gestational diabetes is an especially attractive target for such studies because the disease is detected in the course of routine clinical care and it provides opportunity to study relatively early stages of glucose dysregulation that may be fundamental to the long term pathophysiology of diabetes.

Glucokinase gene encodes for human glucokinase enzyme, a major component of glucose sensing enzyme. The gene exists as single copy in genome which is mapped to chromosome 7p13 [10, 11].

The gene contains 12 exons including two tissue specific exons one active in islet beta cells (1B) and the other active in liver (1H). One optional cassette exon (2A) which was expressed as a minor form in liver [10].The 12 exon are scattered over 16 kilobase(kb) in genome. These exons are interrupted by 9 introns. Glucokinase gene contains two different transcription control regions. One region regulates the transcription of the gene in the liver and the other controls the transcription in the pancreatic beta cell [10].

The prevalence of glucokinase gene mutation in gestational diabetes is 0 to 6%[8]. A misssense mutation was identified in the exon 7 of glucokinase gene in women with gestational diabetes [12]. However there are no studies done of glucokinase gene polymorphism in subjects with gestational diabetes in India [13].

Glucokinase mutations cause a form of maturity onset diabetes of young(MODY) that is MODY 2. It has four criteria [14]

1. Persisting fasting hyperglycemia.
2. Small increase in glucose above fasting level in oral glucose tolerance test.
3. Insulin treatment during at least one pregnancy.
4. A first degree relative with type 2 diabetes 80% women who met all the four criteria had glucokinase mutations.

MATERIALS AND METHODS :

This is a prospective study conducted at Kamineni Hospital LB nagar, Hyderabad. The controls and cases were taken from outpatient and inpatient departments. The subjects were selected from the cosmopolitan population of Hyderabad coming from different religions and different socioeconomic background. 50 pregnant women having gestational diabetes are taken as cases and 50 pregnant women with normal sugar levels taken as controls in this study. The period of study was from April 2015 to April 2017. The cases and controls were subjected to all necessary investigations like urine examination, fasting blood sugar, postprandial blood sugar, oral glucose challenge test, oral GTT, complete blood test, ultra sonogram, Non stress test.All pregnant women at or more than 24 to 28 weeks of gestation with gestational diabetes and those patients with abnormal glucose tolerance test in pregnancy were included in the study. Pregnant women with normal blood sugar levels were taken as controls. Samples have been collected after proper counseling and obtaining informed consent from selected subjects. Under all aseptic precautions 2ml of venous blood is collected into a disposable syringe containing 1.25ul of heparin. The syringe is rotated in all directions so as to assure a proper mixing of blood with heparin. These samples are transferred to genetic laboratory where the DNA was isolated from these samples. Steps involved in processing are – DNA isolation-PCR- Restriction fragment length polymorphism- electrophoresis on poly Acrylamide Gel(PAGE)- Ethidium bromide staining- silver staining.

OBSERVATIONS:

In this study 50 pregnant women with gestational diabetes were taken as cases and 50 pregnant women with normal blood sugar levels were taken as controls as per the inclusion criteria. These women were managed according to the standard protocol of the hospital. They were followed for six weeks period postpartum period. Age of the women in cases was varying from 20 to 40 years and in controls was 15 to 34 years. 44% of women in cases and 8 % of women in controls were of elderly age group that is above 30 years.The mean age for cases was 29.82 years.The mean age for controls was 24.81 years. (Table-1)

Table- 1: The age pattern in the cases and controls

AGE	CASES	CONTROLS
15-19 yrs	Nil	02(4%)
20-24yrs	06(12%)	20(40%)
25-29 yrs	22(44%)	24(48%)
30-34 yrs	17(34%)	04(8%)
35-39 yrs	4(8%)	-
40-44 yrs	1(2%)	-

BODY MASS INDEX (BMI) :
BMI was varied from 18.5 to 40 kg/m2 in cases and in controls 18.5 to 34.99kg/m2. About 22% of women in cases were having BMI above 30 kg/m2 where as only 4% of women in controls were having BMI above 30kg/m2.Obesity is known to be associated with serious obstetric complications. Studies have reported that there is increased incidence of Gestational diabetes mellitus, gestational hypertension, preeclampsia, shoulder dystocia and cesarean section [5].

PARITY:
Above 36% of women in cases were primigravida and in controls 50% of women were primigravida. About 40% of women in cases were second gravid as compared to 32% of women in controls. About 24% of women in cases were multigravida as compared to 18% of women in controls.(Table-2)

Table -2: Parity

Primigravida	18(36%)	25(50%)
Gravida two	20(40%)	16(32%)
Gravida three and above	12(24%)	09(18%)

FAMILY HISTORY OF DIABETES : Family history of diabetes was positive in 58% of cases and it is negative in controls.

GESTATIONAL AGE AT WHICH GDM WAS DIAGNOSED: The mean gestational age at which GDM was diagnosed is 26.75 weeks.

MODE OF DELIVERY : 64% of women in controls had normal vaginal delivery as compared to 16% in cases. 36% in controls had lower segment caesarian section as compared to 84% of women had Lower segment caesarian section in cases.

LIVE BIRTHS: There were 100% of live births in controls as compared to 96% live birth in cases.

INTRAUTERINE DEATH: There was no intrauterine death in controls as compared to 4% of intrauterine death in cases.
AVERAGE BIRTH WEIGHT: Average birth weight seen in controls was 2.7kgs where as in cases it was 3.1kgs

GENE POLYMORPHISM & GENOTYPES:
We have studied hepatic GCK polymorphism in 50 women with GDM as cases and in 50 women with normal pregnancy as controls. The study was done in Indian women. Their genotypes were determined by using PCR_RELP assay. A PCR product of 173bp was obtained which digestion with restriction enzyme fragments of 173bp indicating GG, 173bp and 154bp indicating GA, 154bp indicating AA. These were statistically significant. A single nucleotide polymorphism (SNP), G to A was selected at 258 position of hepatic GCK gene.

GENOTYPES IN CASES AND CONTROLS:
We identified 37(74%) GG genotype 13(26%) GA genotype in cases, 49(98%) GG genotype1(2%) GA genotype in controls. AA genotype was not seen in both cases and controls. The odds ratio is 10.2345 and 95% of CI=5.9573 to 17.5818. P value is 0.0015. The results were statistically significant.(Table-3)

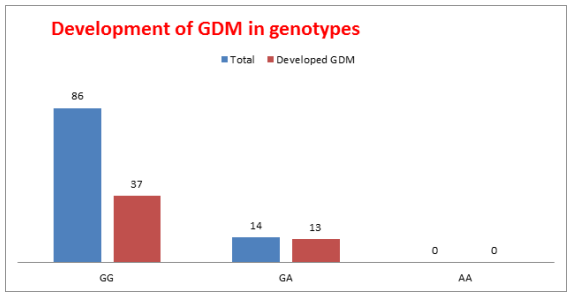
Table -3: Genotype of cases and controls

TOTAL	GG	GA	AA
CASES(n=50)	37(74%)	13(26%)	00
CONTROLS (n=50)	49(98%)	01(2%)	00

DEVELOPMENT OF GDM IN GENOTYPES:
43.02 % of women with GG genotype had developed GDM, where as 92.85% of women with GA genotype developed GDM. This suggests a high association of this genotype in developing GDM.(Table-4)

Table -4: Development of GDM in genotypes

GENOTYPE	TOTAL	DEVELOPED GDM
GG	86	37(43.02%)
GA	14	13(92.85%)
AA	00	00



CLINICAL CHARECTERISTICS OF GENOTYPES:
Women with GG genotype had 43% of positive family history of diabetes mellitus as compared to 100% in women with GA genotype. The onset of disease is at 26.75 weeks of gestation for both the genotypes.13% of women with GG genotype had normal vaginal delivery, as compared to 23% in GA genotype.85% of women with CG genotype delivered by LSCS as compared to 77% of women with GA genotype.65% of women with GG genotype were treated by diet control alone, as compared to none in GA genotype.35% of women with GG genotype were required treatment with insulin as compared to 100% with GA genotype required insulin treatment.

Table -5: Clinical characteristics of genotypes

	GA	GG
NUMBER	13(26%)	37(74%)
FAMILY HISTORY	13(100%)	16(43%)
ONSET GDM	26.75wks	26.75wks
NORMAL VAGINAL DELIVARY	3(77%)	5(13%)
LSCS	10(77%)	32(86%)
DIET CONTROL	NIL	24(65%)
INSULIN	13(100%)	13(35%)

Glucokinase was originally proposed to be a glucose sensor and metabolic signal generator in pancreatic beta cells and hepatocytes. Mutations in the hepatic glucokinase may play a role in the pathogenesis of insulin resistance and type 2 diabetes mellitus[6]. There are two forms of glucokinase liver and islet cells of pancreas. The transcript of islet GCK is regulated by glucose while insulin is the key regulator for hepatic GCK transcription. In this study single nucleotide polymorphism (SNP) was selected at the position 258, G to A polymorphism of the hepatic GCK promoter. It is an independent determinant for hepatic insulin sensitivity [7].Hepatic and whole body insulin sensitivities are well correlated to each other. A better correlation is seen between hepatic insulin sensitivity and this polymorphism. This suggests the primary impact of this polymorphism on hepatic insulin sensitivity.This is the first study where hepatic glucokinase promoter polymorphism is studied in GDM. Hyperglycemia in animals has been shown to decrease hepatic glucokinase activity which can be reversed by treatment with insulin. Over expression of hepatic GCK in mice liver resulted in decreased glucose concentration and reduced body weight [7]. Therefore reduced hepatic GCK expression can lead to hepatic insulin resistance. The G to A polymorphism at 258 nucleotide position of hepatic GCK promoter is associated with hepatic insulin resistance. However the cause effect relationship between this polymorphism and insulin resistance to be elucidated [7].The frequency of GA genotype in cases is 26% and in controls is 2%. The purpose of this study is to determine the glucokinase gene polymorphism as risk factor in development of

GDM. In this study GA genotype is found higher in frequency in cases than control group. The difference is statistically significant (P=0.0015).When comparing with S.Ellard.et al (2000) study in Caucasians he found mutations in 12 subjects out of 15 subjects. That is 80% of mutation. This high percentage is because he had selected the subjects phenotypically that may be the reason for high percentage of mutation [8].

SUMMARY AND CONCLUSION :

We have studied hepatic GCK polymorphism in 50 cases and 50 controls. The study was done in Indian women. Their genotypes were determined by using PCR-RELP assay. A single nucleotide polymorphism at 258, G to A polymorphism is identified in hepatic GCK promoter. We identified 37(74%) GG genotypes, 13(26%) GA genotypes in cases, 49(98%) GG genotypes 1(2%) GA genotype in controls. AA genotype was not seen in both cases and controls. The difference is statistically significant (P=0.0015).This study indicates glucokinase gene polymorphism is definitely a risk factor in developing gestational diabetes. As the odds ratio is 10.2345, those having this mutation are ten times at risk of developing gestational diabetes than the general population. Phenotypic selection increases the chance of detecting the polymorphism in glucokinase gene.

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