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Original Research Paper

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SOCIO-DEMOGRAPHIC, BIOCHEMICAL FACTORS AND EXPRESSION ANALYSIS OF GLUT-4 GENE IN GESTATIONAL DIABETES MELLITUS PATIENTS

Ritambhara Ratnapriya*	*Corresponding Author
Nisha Rani Agarwal	Professor
Dr. Kiran Singh	
Dr. Shikha Sachan	

ABSTRACT

Introduction: Gestational Diabetes Mellitus(GDM),is defined by the American Diabetes Association as "diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes".GDM affects 5% of al pregnancies and results in an increased incidence of Caesarean sections, perinatal traumas and neonatal complications. This study was undertaken to study about the socio-demographic factors, biochemical factors and expression analysis of GLUT-4 gene in placenta of GDM patients. The purpose was to reduce the adverse outcomes by early identification and proper explanation of risks to GDM patients. **Materials and Methods:** Women with deranged sugar levels (RBS>140mg/dl) and normal sugar levels reporting to Antenatal check-up clinic or labour room were enrolled in the study.The study was conducted in 25 cases of GDM and 25 controls which were women of reproductive age group(18-40) years.This study was conducted over a period of 2 years(June2018-June 2020)in SSH-BHU.The study for expression of GLUT-4 gene in placenta of GDM patients was done in department of Human and Molecular Genetics. **Results:** The study was done in 25 cases and 25 controls.Tha mean age of cases was more (29.36 years) than controls(27.36 years) but the p value was not significant.72% of cases had family history of diabetes.68% of cases had BMI of 25-28 while only 28% controls had BMI in this range. Cases had raised serum levels of HbA1c,Cholesterol, triglyceride and insulin.44% of cases had fetal weight between 3.5-4kg.The p value was 0.02 which was statistically significant.Our study has also shown decreased expression of GLUT4 gene in GDM cases as compared to healthy controls,however,the difference was not significant. **Conclusion:** The current study reports that the patients with Gestational Diabetes Mellitus are mostly from lower middle class,age>30 years,obese,have family history of GDM,increased levels of HbA1c and also have fetal macrosomia.The study also reports that increased levels of serum insulin,serum cholesterol, serum triglycerides have a positive correlation with severity of Gestational Diabetes.There is no significant difference in expression of GLUT-4 gene in placenta of gestational diabetes cases and controls which is in co-relation with previous studies.

KEYWORDS : GDM.GLUT-4.OGTT.DIPSI.HAPO.Macrosomia.Insulin.HbA1c.BMI

INTRODUCTION:

Gestational diabetes mellitus,is defined by the American Diabetes Association as "Diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes".WHO has further classified the period of diagnosis as Hyperglycemia in Pregnancy and Gestational Diabetes Mellitus.The former term is applicable in the early period of gestation and GDM is detected after 24 weeks.The prevalence is rising and correlates with the increase in maternal obesity over recent decades.The etiology of GDM is complex,with genetic and environmental factors implicated in mechanistic and epidemiological studies.

A possible cause of GDM is obesity,which is an important clinical risk factor for the development of diabetes.Women who develop GDM generally have higher body mass indices when compared with healthy pregnant women,and obesity can induce low-grade inflammation.Chronic low-grade inflammation induces the synthesis of xanthurenic acid,which is known to be associated with the development of type 2 diabetes,pre-diabetes and GDM.The development of GDM may represent a very early stage of progression to type 2 diabetes that is being manifested under the stresses of pregnancy.

MATERIALS AND METHODS:

The study was conducted in the Department of Obstetrics and Gynaecology, Institute of Medical Sciences,Banaras Hindu University.Written informed consent was taken from the patients or relatives.It was done in 25 cases and 25 controls in patients between 18-40 years of age.This study was carried out for a period of two years(June2018-June2020).It was a prospective,observational study.Patients with age <18 years and >40 years,those with presence of HIV/AIDS and those with known medical disorders like malignancy or significant comorbidity patients with chronic heart disease and renal disease were excluded from study.Women with deranged sugar levels by DIPSI i.e.RBS>140mg/dl and normal sugar levels,reporting to Antenatal check-up clinic or labour room were enrolled in the study. When the patient reported in the antenatal clinic or in the labour room as an emergency case,a detailed history elicited in each case were recorded such as maternal age,parity,socio-economic status,address,family history of diabetes, past history of diabetes,drug history. Indian women have an eleven fold increased risk of developing GDM as compared to Caucasian women,so universal screening is done in India.

At first antenatal visit,screening for undiagnosed type 2 diabetes is done at the first antenatal visit using a standerd diagnostic criteria.

Universal early testing is recommended in populations with high prevalence of type 2 diabetes(IAGDSG).

- At 24-28 weeks:Screening for gestational diabetes mellitus(GDM) is done at 24-28 weeks in all pregnant women not previously known to have diabetes.
- All the data is recorded in a pre-designed proforma.
- The patient examined thoroughly. Obstetrical examination was done.
- Those patient who had uncontrolled sugar levels were admitted in the hospital, and strict 6 point sugar monitoring was done.All patients were explained about the risks of deranged sugar levels.
- Relevant investigations sent including OGTT, Hba1c, serum insulin, serum Cholesterol, serum triglycerides.

For studying the expression of Glut-4 gene in placenta of GDM patients following steps were followed:

Procedure:

1. Homogenized tissue samples (chorionic villi) in TRIZOL reagent.
2. To ensure complete dissociation of nucleoprotein complexes,sample was allowed to stand for 5-10 minutes at room temperature.Then 120μl of chloroform were added.
3. Eppendorf tubes were closed tightly and shaken vigorously or homogenized for 30-60 seconds and allowed to stand for 15 minutes at room temperature.
4. The resulting mixture was centrifuged at 12000xg for 15 minutes at 4c.By Centrifugation mixture is dispersed into three phases-A red organic phase,a Middle interphase and a colourless upper aqueous phase containing RNA.

DNase Treatment was done.

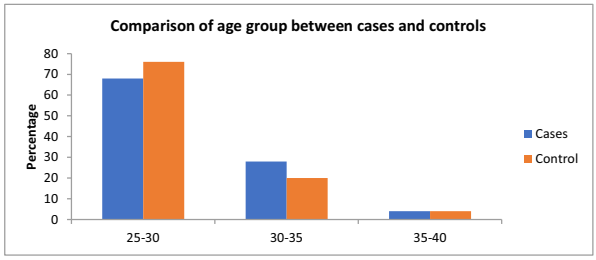
cDNA was prepared.

Comparison of GLUT-4 gene expression in GDM with respect to control

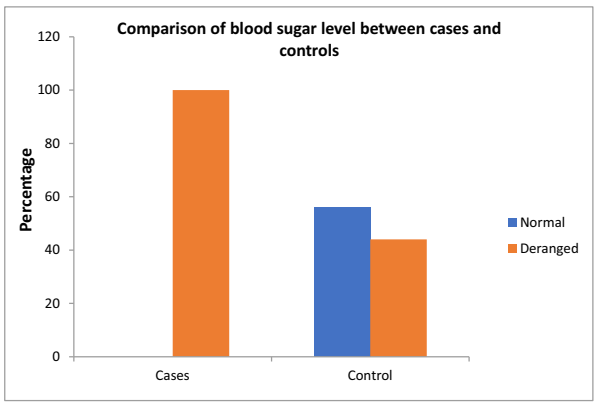
Samples

- The cDNA synthesized from RNA of the placental samples extracted from both
- GDM patients and control were then subjected to quantitative PCR or real time
- PCR amplifications with primers of beta actin and GLUT-4 gene.

RESULTS:

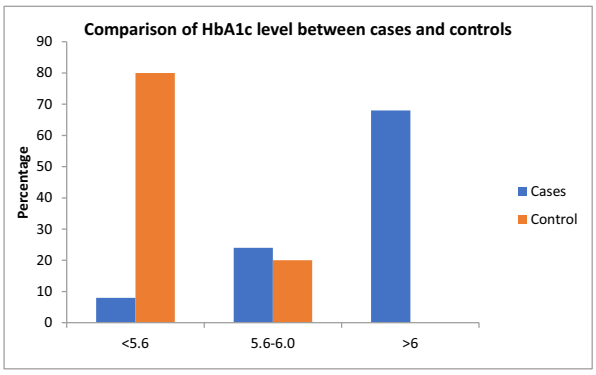


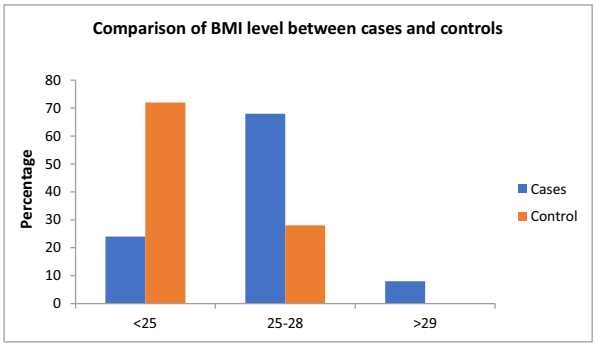
From the above figure, it is shown that in the reproductive age group the mean age of the cases at the time of delivery was 29.36 years with a SD of +2.95.The mean age group of the controls at the time of delivery was 27.36 years with a SD of +2.56. The p value is 0.801 which is not significant.



From the above figure, it is seen that blood sugar level is deranged in almost all cases of GDM, but it is deranged in 44% of controls. The p value is <0.001 which shows that the difference is significant.

In the below table it is seen that 68% of cases had HbA1c more than 6 while none of the control had it. The mean value for cases was 6.8 with a standerd deviation of 0.42 while the mean value for control was 5.3 with a standerd deviation of 0.51. The p value is <0.001 which means that it is significant.





From the above table, it is seen that 68% of cases had BMI of 25-28 and 8% of cases had BMI >29. In controls, 72% had BMI <25 and 28% had BMI between 25-28. The mean value of BMI for cases was 26 with a standard deviation of +2 while the mean value of BMI for controls was 21 with a standard deviation of +1.42. The p value is 0.002 which suggests that the difference is significant.

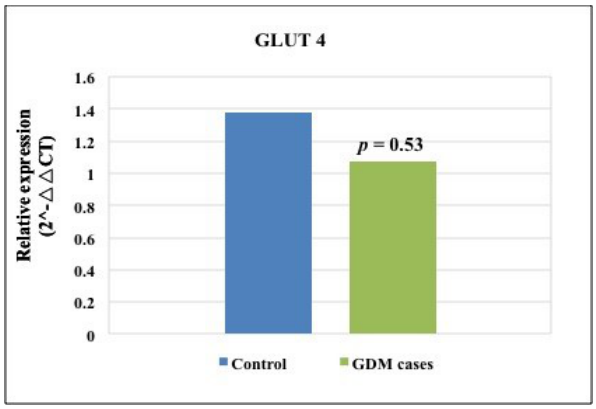


Figure Expression analysis of GLUT 4 gene in placenta of GDM cases vs. controls

From, the above graph we see that there is no significant difference in the expression levels of GLUT-4 gene in cases and control (p=0.53).

Table : Comparison of mean laboratory investigations and mean fetal weight between cases and controls

	Group 1 Mean±SD N=25	Group 2 Mean±SD N=25	p -value
HbA1c	6.8680.42	5.3200.51	<0.001
BMI	26.002.00	21.881.42	<0.001
Cholesterol	199.7212.52	191.769.42	0.014
Triglyceride	155.7612.29	143.566.03	<0.001
Insulin	59.6020.52	22.362.05	<0.001
Fetal weight	3.3760.46	2.7560.38	<0.001

From the above table it is seen that HbA1c, BMI, serum cholesterol, serum triglyceride, serum insulin, fetal weight is more in cases than in controls. The p value is <0.05, which suggests that the difference is significant. The mean values of all parameters are also stated.

DISCUSSION:

In women with gestational diabetes, maternal body mass index (BMI) is an independent and more substantial risk factor for fetal macrosomia than is glucose intolerance (Ehrenberg, 2004; Mission, 2013).

In our study also, the mean BMI for cases was 26 and for controls was 21.The p value was 0.002 which suggests that the difference is significant which concludes that increased BMI is associated with increased risk of GDM.

Twenty obese pregnant patients, ten with GDM and ten non-diabetic control subjects were selected. The two obese groups were evaluated through an oral glucose tolerance test, taking blood at 0, 60, 120 and 180 minutes; after centrifugation glucose serum levels were measured immediately by the glucose oxidase technique and the rest of the sample was kept frozen at -20 ^{0}C until insulin determinations by radioimmunoassay. Insulin serum levels in GDM group were higher than in women without GDM ($p < 0.01$).

In our study, mean value of serum insulin in cases was 59.6IU/l with a standard deviation of +20.52 while mean value of controls was 22.36 with a standard deviation of +2.05.The p value <0.0001 which is considered as significant.

The present study comprised 33 patients with GDM, 33 patients with PE and 33 subjects with normal pregnancy were enrolled from regular checkups performed in the Department of Obstetrics of the Maternity and Child Health Hospital of Zhenjiang (Jiangsu, China) between January 2013 and August 2016

The objective of this study was to investigate the role of blood glucose, lipid metabolism, body mass index (BMI) in the pathogenesis of concurrent gestational diabetes mellitus (GDM) and preeclampsia (PE). Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and fasting blood glucose (FBG) were also detected. The levels of low-density lipoprotein (LDL) were calculated using the Friedewald formula.

Significantly higher serum TG, TC, LDL and very LDL were detected in patients with GDM, PE and DPE compared with those in subjects with normal pregnancies. By contrast, the concentration of HDL was lower in the patient groups.

The accumulation of cholesterol and TGs may cause damage to endothelial function. It has been reported that lipid profile abnormalities in maternal patients with GDM increase the risk of vascular injury, which may lead to endothelial dysfunction, a pathogenic factor of PE.

In pregnant women who are obese, the plasma levels of TG and VLDL are higher, and the concentration of HDL is lower compared with those in non-obese pregnant women. It is possible that in pregnant women who are obese, the HDL concentration may be insufficient to fully protect the maternal vascular endothelium. Obese pregnant women are at a higher risk of endothelial dysfunction.

In our study, mean value of serum cholesterol in cases was 199.72mg/dl and mean value in controls was 191mg/dl. The p value was 0.014 which shows that the difference is significant.
Glycated haemoglobin (HbA1c) is an important tool for assessing glycaemic status in patients with diabetes, but its usefulness in gestational diabetes mellitus (GDM), is unclear. In this study we also tried to evaluate whether HbA1c in women with GDM is valuable in predicting adverse pregnancy out or not.

These studies showed that measurement of HbA1c, either at the time of diagnosis of GDM or toward the end of pregnancy, were both associated with adverse pregnancy outcomes. Women with elevated HbA1c (>5.4% or 35 mmol/mol) at diagnosis of GDM should be monitored closely during pregnancy.

In our study, there is no significant difference in the expression of GLUT-4 gene in placenta of GDM patients. In two to three previous studies also there was no difference in expression, but one study has shown increased expression in placenta of GDM patients especially when they are on insulin therapy. There is also a decrease in the number of insulin receptors as the pregnancy progresses.

CONCLUSION:

The present study reflected the following observations:

1. The patients were in the age group of 25-40 years.
2. Out of 50 patients enrolled for study, 25 were diagnosed as having gestational diabetes.
3. Although the mean age of cases were more than controls. But age is not a significant factor for GDM.
4. Obesity has a direct correlation with the incidence of gestational diabetes mellitus. Mean BMI of cases was 26 and that of controls was 21.88.The p value was 0.002 which shows that the association is significant.
5. Rising level of serum cholesterol and serum triglyceride are associated with gestational diabetes. The p value is less than 0.001 which suggests that there is a significant difference between cases and controls.
6. The mean value of HbA1C in cases are higher than the mean value in control. The p value is <0.001 which suggests that there is a significant association between raised HbA1C levels and incidence of GDM.
7. There is no significant difference in expression of GLUT-4 gene in placenta of GDM patients and controls (p=0.53).

The current study reports that the patients with Gestational Diabetes Mellitus are mostly from lower middle class, age>30years,obese,have family history of GDM, increased levels of HbA1c and also have fetal macrosomia. The study also reports that increased levels of serum insulin, serum cholesterol, serum triglycerides have a positive correlation with severity of Gestational Diabetes.

Results of the current study validate previous studies which support the school of thinking that the incidence of gestational diabetes is associated with obesity, have a family history of diabetes and fetal macrosomia. They also have high levels of HbA1c which shows poor sugar control and is also associated with increased serum cholesterol, serum triglycerides which is seen in patients of pre-eclampsia as well. There is no significant difference in expression of GLUT-4 gene in placenta of gestational diabetes patients and controls which is in co-relation with previous studies.

Serum insulin levels are also increased. Obstetricians dealing with these patients should identify the risk factors and do relevant investigations so that timely management of blood sugar levels can be done to minimize the adverse neonatal complications. It has seen in many studies that uncontrolled sugar levels has resulted in intrauterine death.

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