Research Article
Volume 11:11, 2022

Molecular Biology: Open Access

ISSN: 2168-9547 Open Access

Association of Leptin Gene Polymorphism with Metabolic Parameters in Gestational Diabetes Mellitus: A Cross-Sectional Study

Usha Adiga[1]*, Nandit Banawalikar P[2], Sachidananda Adiga MN[3], Desy TM[1] and Lakshmi Manjeera[4]

[1]*Department of Gynecology, NITTE University, Karnataka, India*
[2]*Department of Biochemistry, NITTE University, Karnataka, India*
[3]*Department of Pharmacology, NITTE University, Karnataka, India*
[4]*Department of Obstetrics and Gynecology, NITTE University, Karnataka, India*

Abstract

Background: Gestational diabetes mellitus (GDM) is a complication of pregnancy that is characterized by impaired carbohydrate tolerance as a result of insulin resistance. Objective was to find association between leptin gene with leptin levels, insulin resistance as well as lipid profile in GDM patients as compared to normal glucose tolerant pregnant women.

Method: In this cross-sectional study, 100 GDM patients and 100 gestational age and BMI-matched healthy pregnant women were included. Genotyping of leptin gene LEPG2548A (rs7799039) was performed by PCR-RFLP. Biochemical parameters were estimated. Various insulin resistance models were constructed using suitable homeostasis model assessment formulae. Chi-square test was used to investigate the associations, Mann Whitney U test to compare biochemical parameters, Spearman's test for correlation studies was used s. Odd's ratio was computed to study the extent of risk of leptin gene polymorphism in causing GDM. 'p' value <0.05 was regarded as statistically significant.

Results: No significant association was observed between leptin gene polymorphism and GDM, leptin levels and insulin resistance. Comparison of IR models among cases and controls showed a significantly low (p<0.0001) HOMA B cell and HOMA 1% B cell (insulin based) as well as significantly high (p<0.0001) HOMA B cell, HOMA 1% B cell (C peptide-based) in cases. It was also observed that C-peptide based insulin resistance models were significantly high (p<0.0001) in cases as compared to controls.

Conclusion: There is no significant association between LEPG2548A alleles with GDM, leptin levels and insulin resistance C–peptide based insulin resistance models were elevated in GDM patients.

Keywords: Leptin • Gene polymorphism • Leptin gene • Insulin resistance • Gestational diabetes

Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance of varying severity with onset or first recognition during pregnancy. The prevalence of GDM is 7% of all pregnancies in the world. However, a recent study reported a prevalence of 9% in India. GDM develops as a result of decreased insulin sensitivity and results in altered metabolic effects like increased postprandial FFAs, increased hepatic glucose production and high blood glucose levels. Leptin is an important adipokine, mediating lipid and carbohydrate metabolisms, insulin sensitivity, atherosclerosis, angiogenesis, etc. Leptin levels are reported to be altered, may be increased or decreased in GDM. Reports available being conflicting and the facts are yet to be established. Insulin resistance in GDM has been associated with elevated leptin levels. As leptin is closely associated with lipid metabolism, it may be contributing to dyslipidemia in GDM [1].

***Address for Correspondence:** Usha Adiga, Department of Gynecology, NITTE University, Karnataka, India, E-mail: ushachidu@nitte.edu.in*

Received: 02 November, 2022, Manuscript No: MBL-22-55081; **Editor assigned:** 04 November, 2022, PreQC No: P-55081; **Reviewed:** 15 November, 2022, QC No: Q-55081; **Revised:** 20 November, 2022, Manuscript No: R-55081; **Published:** 28 November, 2022, DOI: 10.37421/2168-9547.2022.11.354

Gene polymorphisms in GDM

Various studies have reported the influence of gene polymorphisms on the causation of GDM. A study by Hofstadter *et al* reported an association between LEP G2548A polymorphism and elevated leptin levels. Yang *et al* studied both the leptin gene and its receptor polymorphism in the Chinese population. There are no such reports available in the Indian population. Clinical study reports suggest that elevation of leptin levels is due to up-regulation of the leptin gene due to insulin resistance and hyperinsulinemia. It has been reported that leptin affects whole-body insulin sensitivity by regulating insulin-mediated glucose metabolism by skeletal muscle as well as hepatic regulation of gluconeogenesis. Leptin was found to have an inhibitory effect on insulin secretion. A study reported elevated leptin levels associated with a high TG/HDL-C ratio. Altered leptin levels along with insulin resistance in GDM patients may alter their lipid levels. Insulin resistance is the key factor in the development of GDM [2]. Reduced maternal pregravid insulin sensitivity coupled with inadequate insulin response is the causative factor for GDM.

Objectives of the study were

1. Evaluate the pattern of polymorphism of leptin gene (LEPG2548A)in GDM and find its association with serum levels of leptin
2. Compare leptin and other biochemical parameters in GDM patients and normal pregnant women
3. Find the association between leptin gene polymorphism with insulin, leptin, insulin resistance and lipid profile in gestational diabetes mellitus.

Methodology

Study design and setting

This cross-sectional study was conducted in the Endocrinology and molecular genetics wing of Central Research Laboratory of K.S. Hegde Medical Academy and Department of OBG, K.S. Hegde Charitable Hospital of Nitte University, Mangaluru, Karnataka, India. University ethics committee approval was sought before starting the study (Ref: NU/CEC/2018/01).

Study subjects

Inclusion Criteria for cases and control: One hundred GDM patients diagnosed as per American Diabetic Association criteria 2017 who are willing to participate in the study were included. Hundred gestational age and BMI matched normal glucose tolerant pregnant women were recruited as a control group.

Exclusion Criteria for cases and control: Cases of multiple pregnancies, known pre-gestational diabetes, pregnancies complicated by major fetal malformations or known major cardiac, renal or hepatic disorders, pregnancy-induced hypertension were excluded [3].

Preformat containing general information on demographic characteristics, parity, family history of diabetes and hypertension and history of GDM, were obtained to check whether the patient fulfills pre-determined criteria. Central Ethics committee (NU) approval was obtained before starting the study. A patient information sheet (in their vernacular language) was given to the eligible patients and explained the aim, procedure and their rights and role in the study. Enough time was given to them to arrive at the decision. Written Informed Consent was taken from patients.

Collection and analysis of blood sample

Five milliliters of venous blood samples were drawn from the recruited patients for biochemical and genetic analysis after obtaining written informed consent from patients. Two ml of blood was collected in a plain vial was used for fasting leptin, insulin, C-Peptide and lipid profile. Fasting leptin, insulin and C-peptide were assayed by ELISA. Lipid profile and fasting blood sugar were analyzed using a fully automated chemistry analyzer, CobasC311. Insulin resistance was calculated by the homeostasis model assessment (HOMA) model. Both insulin and C-peptide-based insulin resistance models were constructed using appropriate formulae for HOMA-IR, HOMA B cell and HOMA -1% B cell, denoted by HOMA-IRC, HOMA B cell -C and HOMA 1% B cell -C for C peptide-based insulin resistance.

Genetic analysis

Three ml of venous blood sample, collected in EDTA (2%) vial was utilized for DNA extraction and genotyping. DNA was extracted from leukocytes by using a DNA extraction mini kit. The quality of the DNA was checked by electrophoresis on 0.8% Agarose gel, containing ethidium bromide (0.5µg/ml) in TAE buffer. The quantification and purity of DNA were checked by the spectrophotometer (ratio of OD260 / OD280). DNA concentration was calculated using the following formula:

Concentration (µg/ml) of DNA in original solution= Absorbance x 100 x 50 µg/ml

Genotyping was carried out by PCR-RFLP. The PCR was carried out using suitable forward and reverse primers for leptin, LEP G2548A alleles. The final product was digested with suitable restriction enzymes [4]. The reaction mixtures were electrophoresing on 2% agarose gel and visualized by ethidium bromide staining. Details of primers and restriction enzymes used are depicted.

Data availability statement

The data may be shared on approval of the funding agency.

Statistical analysis

The statistical analysis was carried out with SPSS 23.0. Categorical data were expressed as percentages and continuous data was expressed as mean ± standard deviation (SD). Hardy-Weinberg Equilibrium (HWE) for the LEP gene variant among cases was performed and a chi-square test was used to compare the distribution of the allele frequencies between different variants. A Chi-square test was used to find the association between genotype distribution and serum concentration of leptin, insulin resistance. Mann Whitney U test was used to compare biochemical parameters between cases and controls [5]. Spearman's correlation test was used to find the correlation between biochemical parameters and insulin resistance. Odd's ratio was computed to study the extent of risk of leptin gene polymorphism in causing GDM. A 'p' value <0.05 was regarded as statistically significant.

Results

The patient characteristics (age, body mass index and gestational age were given in indicating that the groups are comparable. GDM patients with a mean age of 29.62 ± 4.3 yrs and normal glucose tolerant pregnant women with 27.08 ± 3.73 yrs were included in the study. The mean BMI of the groups was 25.78 ± 6.84 kg/m^2 and 25.86 ± 5.86kg/m^2 respectively. The gestational age of the subjects was 25.87 ± 1.21 wks and 26.1 ± 1.54 wks respectively.

Leptin gene polymorphism pattern

The distribution of genotypes and alleles of LEP (rs7799039). None of the genotype frequency distributions for rs7799039 variants deviated significantly from HWE in GDM cases (P>0.05), suggesting that alleles were in equilibrium.

Association studies between the leptin gene and GDM were carried out. Chi-square statistic with Yate's correction was 0.1694 and p=0.68 for the association between leptin gene polymorphism and GDM, suggesting no significant association between them. However, Odd's ratio showed that individuals with the A allele were at 1.25 times higher risk of developing GDM.

No significant association was observed between the LEP gene polymorphisms and leptin levels with Chi-square statistic and Yates correction being 0.0626 (P=0.802). It was also observed that there was no significant association between leptin gene polymorphisms and insulin resistance (Chi-square statistic =0.805, p=0.369). However, subjects with homozygous dominant AA allele were at higher risk of (1.25 times) developing GDM. Odd's ratio showed 1.4 times the risks of IR in patients with the 'A' allele for the leptin gene.

Biochemical parameters and Insulin resistance

Fasting blood sugar and fasting C peptide levels in cases were significantly higher in comparison to control ((p<0.0001, p=0.0014 respectively). Fasting serum insulin and leptin levels were insignificantly low in GDM patients (p=0.6968 and p=0.213). There was no significant difference between lipid profile parameters like TG, TC, HDL, LDL and VLDL (p-values being 0.343, 0.091, 0.57, 0.61, 0.65, 0.097, 0.157, 0.12 respectively) levels between cases and controls.

Comparison of IR models among cases and controls showed a significantly low (p<0.0001) HOMA B cell and HOMA 1% B cell (insulin based) as well as significantly high (p<0.0001) HOMA B cell, HOMA 1% B cell (C peptide-based) in cases. It was also observed that C peptide based insulin resistance models (HOMA IR-C and CIR) were significantly high (p<0.0001) in cases as compared to cases. However, there was no significant difference in insulin-based HOMA IR and QUICKI, between cases and controls.

Biochemical parameters and Insulin resistance with leptin gene polymorphism

On comparing biochemical markers among cases with different genotypes of leptin gene, AA, AG and GG, no significant difference in Insulin, C peptide, leptin, TG, TC, HDL, LDL and VLDL were observed. (p values being 0.343, 0.091, 0.57, 0.61, 0.65, 0.097, 0.157, 0.12 respectively).

Different IR models, both insulin and C peptide-based, HOMA IR, HOMA B cell, HOMA 1%B cell, QUICKI, HOMA IRC, HOMA B cell- C, HOMA 1%B cell

-C and CIR in different genotypes of leptin didn't vary significantly (p values being 0.402, 0.946, 0.912, 0.99, 0.074, 0.32, 0.17, 0.07 respectively).

Discussion

The present study was undertaken to evaluate the pattern of leptin gene polymorphism in GDM patients and to find its association with IR, fasting insulin, sugar, leptin insulin resistance and lipid parameters.

The frequency of homozygous dominant AA alleles (wild allele) was found to be higher than the homozygous recessive GG allele (mutant allele) for *LEP* G2548A polymorphism. Taiwanese study by Wang *et al also* reported such a pattern of AA allele predominance in GDM patients. But the contradictory reports have been suggested in different populations in which predominance of GG allele frequency was noted. However, there is a scarcity of studies that explored the association of *LEP* G2548A polymorphism in GDM. A Czech study concluded that GDM patients with AA and AG genotypes were significantly associated with GDM as compared to those with GG genotype [6]. But the smaller sample size was the limitation of the study. Our present study didn't show a significant association between LEP G2548A polymorphism in GDM.

Gene polymorphism and leptin levels

The study results confirmed the non-significant association between leptin gene polymorphism and leptin levels. Studies reported a strong association between *LEP* G2548A polymorphism and the expression of this gene. These studies do not agree with the results in the Egyptian study. A study opined that subjects with AA and AG genotypes, due to their higher transcriptional activity of the LEP gene, have a significantly higher risk of gestational diabetes mellitus against those carrying the GG genotype. The study findings support the probable role of leptin in the etiopathogenesis of GDM.

In our study, no significant difference was observed in the serum leptin levels between GDM patients and subjects with normal glucose tolerance. When leptin levels were compared among GDM patients with different genotypes, AA, AG and GG, even though insignificant, patients with the 'AA' allele had the highest serum leptin levels. However, hyperleptinemia was observed in both cases and controls, considering 2.5-21.8 ng/ml as the normal reference range for serum leptin [7].

The altered leptin levels in pregnancy could be due to increased adiposity and placental influence. As leptin has an important role in maintaining maternal glucose homeostasis, it may be considered an important marker in the prediction of GDM. However, the reports available on the levels of maternal leptin in GDM are conflicting. Studies have reported elevated leptin levels in GDM, diminished leptin levels or insignificant differences in leptin levels among GDM patients compared to controls. A study suggested insignificant alterations in leptin levels at 2nd trimester, decreased leptin concentrations at the 3rd trimester in patients with GDM. In a cohort study reported a 20% increase in the risk of GDM, with the 10ng/ml increase in the leptin levels in early pregnancy. Along with plasma leptin levels, its level in the placenta was also increased in GDM patients. These findings suggest that polymorphisms of leptin and its receptor gene could result in altered expression of their proteins [8].

In a case-control study, found that plasma leptin levels were elevated in GDM cases in the third trimester as compared with the normal controls. Similar results were also reported. A correlation was reported between glucose tolerance and serum leptin levels during pregnancy. A case-control study after adjusting for BMI and insulin concentrations, as they may be confounding factors, reported low leptin concentrations in the third trimester in GDM cases.

Pregnancy may be a leptin-resistant state, associated with altered leptin signaling. One possible function of increased maternal leptin levels is to increase availability, support trans-placental transfer of lipid substrates by enhancing the mobilization of maternal fat stores [9]. There is strong evidence that suggests the main contributor of plasma leptin is the placenta rather than the adipose tissue. The human placental promoter region might be differently regulated compared to adipose tissue.

Most studies have found increased leptin concentrations in GDM. Moreover, hyperleptinemia in early pregnancy, independent of maternal adiposity appears to be predictive of an increased risk to develop GDM later in pregnancy.

In our study, no significant difference was observed in serum insulin levels between cases and controls, but C-peptide was significantly higher (p=0.0014) among cases. On comparing among cases with different genotypes of leptin gene, AA, AG and GG, no significant difference in Insulin and C peptide levels (p=0.343 p=0.091) were observed. There was no significant difference in C-peptide levels among various genotypes.

Insulin resistance and leptin gene polymorphism

Comparison of IR models among cases and controls showed significantly low insulin-based IR models, HOMA B cell and HOMA 1% B cell in cases as well as significantly high C peptide-based IR models (HOMA B cell C, HOMA 1% B cell C) were observed in our study. It was also observed that C peptide-based insulin resistance models (HOMA IR -C and CIR) were significantly high (p<0.0001) in cases as compared to cases. Since it is a well-established fact that C-peptide is a better marker of endogenous insulin secretion and the C-peptide-based IR model is a better indicator of insulin resistance, we can conclude that GDM patients have higher IR compared to normal glucose tolerant pregnant women.

The inhibitory effect of leptin on insulin gene expression and its secretion was reported by various previous studies. Leptin was found to bring about inhibition of phosphorylation of glucose transporter 2 (GLUT2) and thereby impairing the transport of glucose tissues [10].

Insulin secretion increases as the gestation progress, reaching the highest levels in the third trimester. Ryan *et al* reported elevated insulin resistance in GDM cases. Leptin influences pancreatic β-cell gene expression and leads to a decline in the secretion of insulin. In addition, leptin influences the proliferation, apoptosis and cell growth of β-cells. Leptin also suppresses the expression of mRNA of insulin in pancreatic β-cells.

Higher leptin levels were noted in impaired fasting glycemic (IFG) compared to the normal glucose tolerant (NGT) group. Similarly, significantly higher fasting insulin levels, HOMA-IR and lower QUICKI were also noted in IFG and IGT groups. Positive and negative correlations were found between plasma leptin levels and HOMA-IR and QUICKI respectively. These correlations were confirmed in some studies but contradicted in a few studies [11]. A positive correlation was observed between plasma leptin concentrations and the pre-pregnant BMI in the GDM group and NGT group.

Lipid parameters and gene leptin polymorphism

There was no significant difference between lipid profile parameters like TG, TC, HDL, LDL and VLDL levels between cases and controls. A significant positive correlation was observed between leptin and TG, TC and VLDL levels among GDM patients. A significant negative correlation was noted between leptin levels and insulin, insulin-based IR models, HOMA IR, HOMA B cell, HOMA 1%B cell and QUICKI among insulin-resistant GDM patients.

Dyslipidemia associated with pregnancy is a well-documented Concept. Lipid levels show a decline in the first trimester but gradually increase later. The elevated estrogen levels and insulin resistance may be contributing to the hypertriglyceridemia associated with pregnancy. An elevation in HDL cholesterol levels was observed at 12 wk of gestation as a result of altered estrogen and remains high throughout pregnancy. Total and LDL cholesterol levels show a decline in early pregnancy but get heightened in the second and third trimesters [12].

GDM induces a state of dyslipidemia in response to insulin resistance. GDM patients were reported to have higher triacylglycerides and lower LDL-cholesterol levels compared to normal pregnant women. A study reported an insignificant difference in total cholesterol, HDL cholesterol and apolipoprotein levels in GDM patients compared to controls.

It was observed in the present study that there was no significant association between leptin gene polymorphisms and insulin resistance. However, Odd's ratio showed 1.4 times the risks of IR in patients with A allele

for leptin genes. Biochemical parameters were compared between insulin resistant cases (HOMA IR>2.4) compared to GDM patients with normal insulin sensitivity. Serum C peptide, TG and VLDL were significantly higher in IR cases.

Studies have demonstrated a positive correlation between adiposity and plasma leptin concentrations. Leptin increases with the changes in maternal fat stores and glucose metabolism in pregnancy. Maternal leptin levels get elevated by 2-3times with the peak around 28 weeks of gestation. An epidemiological study has concluded that plasma leptin concentrations were positively associated with insulin resistance in men and non-pregnant women.

Conclusion

It is concluded from the study that there is no significant association between LEPG2548A alleles and gestational diabetes, leptin levels, insulin resistance. C–peptide based insulin resistance models were elevated in GDM patients. The study could establish a cycle, gene polymorphism altering leptin levels which in turn altering insulin secretion and insulin resistance, contributing to dyslipidemias of pregnancy as well as gestational diabetes.

Acknowledgments

Our **s**incere thanks to Dr. Suchetha Kumari, In-charge of Molecular Genetics Laboratory for the generous support in carrying out the work.

Funding

Research Society for the Study of Diabetes in India (RSSDI New-Delhi)

Conflicts of Interest

None.

References

1. Metzger, Boyd E., Thomas A. Buchanan, Donald R. Coustan and Alberto De Leiva, et al. "Summary and recommendations of the fifth international workshop-conference on gestational diabetes mellitus." *Diabetes care* 30 (2007): S251-S260.
2. Choudhary, Naiyma, Mohd Rasheed and Vivek Aggarwal. "Prevalence of gestational diabetes mellitus, maternal and neonatal outcomes in a peripheral hospital in North India." *Int J Res Med Sci* 5 (2017): 2343-2345.
3. McLachlan, Kylie A., David O'Neal, Alicia Jenkins and Frank P. Alford. "Do adiponectin, TNFα, leptin and CRP relate to insulin resistance in pregnancy? Studies in women with and without gestational diabetes, during and after pregnancy." *Diabetes Metab J* 22 (2006): 131-138.
4. Festa, A., N. Shnawa, W. Krugluger and P. Hopmeier, et al. "Relative hypoleptinaemia in women with mild gestational diabetes mellitus." *Diabet Med* 16 (1999): 656-662.
5. Hoffstedt, J., P. Eriksson, S. Mottagui-Tabar and P. Arner. "A polymorphism in the leptin promoter region (-2548 G/A) influences gene expression and adipose tissue secretion of leptin." *Horm Metab Res* 34 (2002): 355-359.
6. Rossetti, Luciano, Duna Massillon, Nir Barzilai and Patricia Vuguin, et al."Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action." *J Biol Chem* 272 (1997): 27758-27763.
7. Ceddia, Rolando B., Heikki A. Koistinen, Juleen R. Zierath and Gary Sweeney. "Analysis of paradoxical observations on the association between leptin and insulin resistance." *FASEB j* 16 (2002): 1163-1176.
8. Lekva, Tove, Annika Elisabet Michelsen, Pål Aukrust and Tore Henriksen, et al. "Leptin and adiponectin as predictors of cardiovascular risk after gestational diabetes mellitus." *Cardiovasc Diabetol* 16 (2017): 1-10.
9. Wang, Tsu-Nai, Meng-Chuan Huang, Wen-Tsan Chang and Albert Min-Shan Ko, et al."G-2548A polymorphism of the leptin gene is correlated with extreme obesity in Taiwanese aborigines." *Obesity* 14 (2006): 183-187.
10. Vaškù, Julie Anna Bienertová, Anna Vaškù, Zuzana Dostálová and Petr Bienert. "Association of leptin genetic polymorphism-2548 G/A with gestational diabetes mellitus." *Genes Nutr* 1 (2006): 117-123.
11. Furusawa, Takuro, Izumi Naka, Taro Yamauchi and Kazumi Natsuhara, et al. "The serum leptin level and body mass index in Melanesian and Micronesian Solomon Islanders: focus on genetic factors and urbanization." *Am J Hum Biol* 23 (2011): 435-444.
12. Suriyaprom, Kanjana, Rungsunn Tungtrongchitr and Kittisak Thawnasom. "Measurement of the levels of leptin, BDNF associated with polymorphisms LEP G2548A, LEPR Gln223Arg and BDNF Val66Met in Thai with metabolic syndrome." *Diabetol Metab Syndr* 6 (2014): 1-9.

How to cite this article: Adiga, Usha, Nandit Banawalikar P, Sachidananda Adiga MN and Desy TM, et al. "Association of Leptin Gene Polymorphism with Metabolic Parameters in Gestational Diabetes Mellitus: A Cross-Sectional Study." *Mol Bio* 11 (2022):354.