Study Title: **Association of Single Nucleotide Polymorphism of Leptin Receptor Gene with Metabolic Parameters in Gestational Diabetes Mellitus**

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**Title: Association of Single Nucleotide Polymorphism of Leptin Receptor Gene with Metabolic Parameters in Gestational Diabetes Mellitus**

**Abstract:**

**Background:** Genetics plays a major role in the pathophysiology of GDM. Leptin and its receptor genes might have a significant contribution in the disease. Objective of the study is to find the association of leptin gene polymorphism with gestational diabetes mellitus (GDM) and its role in altered leptin levels, insulin resistance and dyslipidemia in GDM.

**Methods:** Hundred GDM patients fulfilling the study criteria, hundred gestational age and BMI matched normal glucose tolerant pregnant women were considered as control group was recruited and five milliliters of venous blood samples were drawn from them for biochemical and genetic analysis. Genotyping of leptin receptor (LEPR)Gln223Arg was performed by PCR-RFLP. Fasting blood sugar, leptin, insulin-peptide and lipid profile were done. Various insulin resistance models were constructed using suitable formulae. The statistical analysis was carried out with SPSS 23.0. Hardy-Weinberg Equilibrium (HWE) for the LEPR gene variant among cases was performed and comparisons of the distribution of the allele frequencies between different variants were carried out using chi-square test. Chi-square test was used to investigate the association between genotypes distribution and serum concentration of leptin and insulin resistance. Mann Whitney U test was used to compare biochemical parameters between cases and controls. Spearmann’s correlation test was used to find the correlation between biochemical parameters. ROC curves were constructed to assess whether leptin levels and IR models can be used as markers to predict GDM.

**Results:** There was no significant association found between leptin receptor gene polymorphism and leptin levels, insulin resistance in GDM. However, Odd’s ratio showed that individuals with A allele were at 1.25 times higher risk of developing GDM. HOMA B cell significantly varied among LepR genotypes (p<0.0001), values being double in AA genotype, compared to AG (p<0.05), 10 times higher in AA compared to GG (p<0.0001).The value was four times higher in AG compared to GG (p<0.01). None of the genotype frequency distributions for rs7799039 and rs1137101 variants deviated significantly from HWE in GDM cases (P>0.05), suggesting that alleles were in equilibrium.

**Conclusion:** It could be concluded from the study that, there is no significant association between leptin receptor, LEPR Gln223Arg alleles and gestational diabetes, leptin levels and insulin resistance. However, subjects with ‘G’ allele for LEPR at higher risk of hyperleptinemia. C –peptide based insulin resistance models were elevated in GDM patients

The study is able to establish a cycle of gene polymorphism altering leptin levels which in turn can alter insulin secretion and insulin resistance, contributing for dyslipidemia of pregnancy as well as gestational diabetes

**Introduction:**

Gestational diabetes mellitus (GDM) is a complication of pregnancy which is characterized by impaired carbohydrate tolerance with onset or first recognition during pregnancy [1]. It develops as a result of decreased insulin sensitivity and results in altered metabolic effects like increased postprandial FFAs, increased hepatic glucose production high blood glucose levels.

Adipose tissue acts as an endocrine gland, produce various adipokines which help in establishing communication between adipose tissue and other organs. Leptin is an important adipokine, mediating a wide range of functions like lipid and carbohydrate metabolism, insulin sensitivity, atherosclerosis, angiogenesis etc. Leptin levels are reported to be altered, may be increased or decreased in GDM [2, 3]. However, reports available are conflicting and fact is yet to be established. Insulin resistance in GDM has been associated with elevated leptin levels [4].As leptin is closely associated with lipid metabolism, it may be attributed dyslipidemia in GDM. As leptin has a wide range of metabolic roles, it may have an impact on pregnancy outcomes, both maternal as well as fetal.

Pregnancy complicated with GDM may have higher risk of miscarriage, hypertensive disorders, macrosomia, operative delivery and postpartum hemorrhage and patients may develop diabetes mellitus in future [5]. The offsprings may be large for gestational age or associated with premature birth, neonatal respiratory distress syndrome, hypoglycemia, and also impaired glucose metabolism in early age [6].

**Objectives of the Study:**

Theobjectives of the study were,

1. to evaluate pattern of single nucleotide polymorphism of leptin receptor LEPRGln223Arg in GDM and to find its association with serum levels of leptin
2. find the association between leptin receptor gene polymorphism and insulin levels as well as insulin resistance in gestational diabetes
3. find the association between polymorphism of leptin receptor gene and lipid profile
4. find the association between the LepR gene polymorphism and pregnancy outcome in terms of the birth weight of the babies

**Methodology:**

**Study setting:** Thestudy was conducted inCentral Research Laboratory of K.S. Hegde Medical academy and Department of OBG, K.S. Hegde Charitable Hospital of NITTE University, Mangaluru, Karnataka, India.

This was a collaborative study which included Department of Biochemistry, OBG and Pharmacology.

**Study subjects**:

Hundred GDM patients diagnosed based on 75 gm oral GTT (OGTT) as per ADA 2016 criteria were taken as cases. Hundred gestational age and BMI matched normal glucose tolerant pregnant women were considered as control group.

**Exclusion criteria**: multiple pregnancies, known pre-gestational diabetes, pregnancies complicated by major fetal malformations or known major cardiac, renal or hepatic disorders, PIH

**Type of study:** Observational Cross sectional

**Timeline**: 2 years

**Ethical Issue:** Institutional Ethics committee approval was obtained (NU/CEC/2018/01) and Written Informed Consent was taken from patients.

**Methodology:**

Patient who are fulfilling the study criteria was recruited. Five milliliters of venous blood samples were drawn from the recruited patients for biochemical and genetic analysis after obtaining written informed consent from patients.

1. **Laboratory Investigations:**

Blood sample collected in 2 ml plain vial were used for biochemical parameters, leptin, insulin, C-Peptide and lipid profile. Fasting leptin, insulin and C-peptide were assayed by ELISA. Lipid profile fasting blood sugar was analyzed using fully automated chemistry analyzer, CobasC311.

Insulin resistance was calculated by homeostasis model assessment (HOMA) model. Both insulin and C-peptide based insulin resistance models were constructed using following formulae;

Table 1: Insulin resistance Models

|  |  |
| --- | --- |
| HOMA –IR | (fasting glucose x fasting insulin)/22.5 ; insulin expressed in μ U/L, glucose in mmol/l. |
| HOMA B cell | 20x insulin / (Fasting blood glucose -3) ; FBS in mmol/l |
| HOMA B 1% | 20x Insulin/ Fasting Plasma Glucose- 3.5 ; FBS in mmol/l |
| QUICKI | 1/ (log G+ log I) |
| C-peptide insulin resistance, CIR | 20/ (Glucose X C-Peptide) ; glucose and C-peptide in mmol/L |

**B. Genetic analysis:**

**Blood sample collection for genetic analysis:** 3 ml of venous blood samples was collected in EDTA (2%) vial. EDTA blood was utilized for DNA extraction and genotyping. DNA was extracted from leukocytes by using DNA extraction mini kit.

**Quality analysis of the extracted DNA:** The quality of the DNA was checked by electrophoresis on 0.8% Agarose gel, containing ethidium bromide (0.5µg/ml) in TAE buffer.

**Quantification of genomic DNA:** The quantification and purity of DNA was 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.*

**Amplification and Genotyping of the gene polymorphism**: Genotyping of all genes was carried out by PCR-RFLP.

**Amplification and Genotyping of LEPR:**  The PCR was carried out using suitable forward and reverse primers for leptin receptor, LEPR Gln223Arg alleles. The final product was digested with suitable restriction enzymes. The reaction mixtures were electrophoreses on 2% agarose gel and visualized by ethidium bromide staining. Details of primers and restriction enzyme used are depicted in table 1.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **SNP** | **Location(Base change)** | **Forward Primer**  **Reverse Primer** | **PCR Program (35 cycles)** | **PCR Fragment length**  **(Bp)** | **Restriction enzyme, Incubation temperature** | **Allele:RFLP fragment size** |
| **LEPR(rs1137101)** | Exon 6(A>G) | 5’-AAACTCAACGACACTCTCCTT-3’  5’-GAACTGACATTAGAGGTGAC-3’ | 93°C,45’,  57°C,30’,72°C,30’ | 80 | MspI,  37 °C | Allele A:80  Allele G:59+21 |

Table 2: Information of PCR-RFLP for the gene Leptin &LEPR

A proforma containing general information on demographic characteristics, socio-economic status, education level, parity, family history of diabetes and hypertension and past history of GDM, were obtained.

**Sample size calculation**: ***-*** There are not many such studies in the literature so far estimating the correlation of gene and gene polymorphisms in the Indian population. However, by extracting the information derived from the studies published so far on LEPG2548Aand taking the prevalence of GDM to be 9.0%, we would require a sample size of 131 patients to design a study with 4% absolute precision and 95% confidence. Due to financial reasons, we restrict the sample size to 100 each for cases and control.

**Statistical analysis**

The statistical analysis was carried out with SPSS 23.0. Categorical data was expressed as percentages and continuous data was expressed as mean ± standard deviation (SD). Hardy-Weinberg Equilibrium (HWE) for the LEPR gene variant among cases was performed and comparisons of the distribution of the allele frequencies between different variants were carried out using chi-square test. Chi-square test was used to investigate the association between genotypes distribution and serum concentration of leptin and insulin resistance. Mann Whitney U test was used to compare biochemical parameters between cases and controls. Spearmann’s correlation test was used to find the correlation between biochemical parameters. 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.Chisquare test was done for the association of gene polymorphism and birth weight of the babies. ROC curves were constructed to assess whether leptin levels and IR models can be used as markers to predict GDM.

**Results**

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. Mean BMI of the groups were 25.78 ± 6.84 kg/m2 and 25.86 ± 5.86kg/m2 respectively. Gestational age of the subjects was 25.87± 1.21 wks and 26.1±1.54 wks respectively. There was also no significant association between leptin receptor gene polymorphism and GDM, with chi-square statistic with Yate’s correction 0.3096 and p=0.577. However, Odd’s ratio showed that individuals with A allele were at 1.25 times higher risk of developing GDM.

No significant association was observed between the LEPR gene polymorphisms and leptin levels, with Chi-square statistic with Yate’s correction being 0.0626 and 0.742 respectively (P=0.802 and P=0.388 respectively). However, subjects with ‘G’ allele for LEPR gene showed twice the risk of hyperleptinemia. It was also observed that there was no significant association between leptin receptor gene polymorphisms and insulin resistance (chi-square statistic =0.1419 p=0.706). Odd’s ratio showed 1.2 times risks of IR in patients with A allele for leptin receptor genes.

The distribution of genotypes and alleles LEPR (rs1137101) gene variants in GDM patients are provided in table 3. None of the genotype frequency distributions for rs7799039 and rs1137101 variants deviated significantly from HWE in GDM cases (P>0.05), suggesting that alleles were in equilibrium.

Table 3: Hardy-Weinberg Equilibrium (HWE) for the LEPR gene

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene variant | | Frequency of pattern in LEP gene (%) (rs1137101) | | Chi -square value |
| Cases | Controls |  |
| AA | Observed | 27 | 25 | 1.94  (Cases)  3.15 (Control) |
| Expected | 23.5 | 20.39 |
| AG | Observed | 43 | 46 |
| Expected | 49.95 | 55.2 |
| GG | Observed | 30 | 42 |
| Expected | 26.5 | 37.38 |
| Association between GDM and LEPR Gene polymorphism | | | | 0.3096  p=0.577 |

**AA:**Homozygous dominant, AG:Heterozygous,GG:Homozygous Recessive

**LEP Receptor: Cases:**Frequency range – ‘A’ allele -0.48; ‘G’ allele – 0.515

**Controls**: p allele frequency: 0.42, q allele frequency-0.58

Comparison of their biochemical parameters showed significantly high (p<0.0001) FBS in GDM cases. Fasting C peptide also was significantly higher in cases (p=0.0014). 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 levels between cases and controls (p=0.069, p=0.12, p=0.73, p=0.255 and p=0.06 respectively) (fig 1).

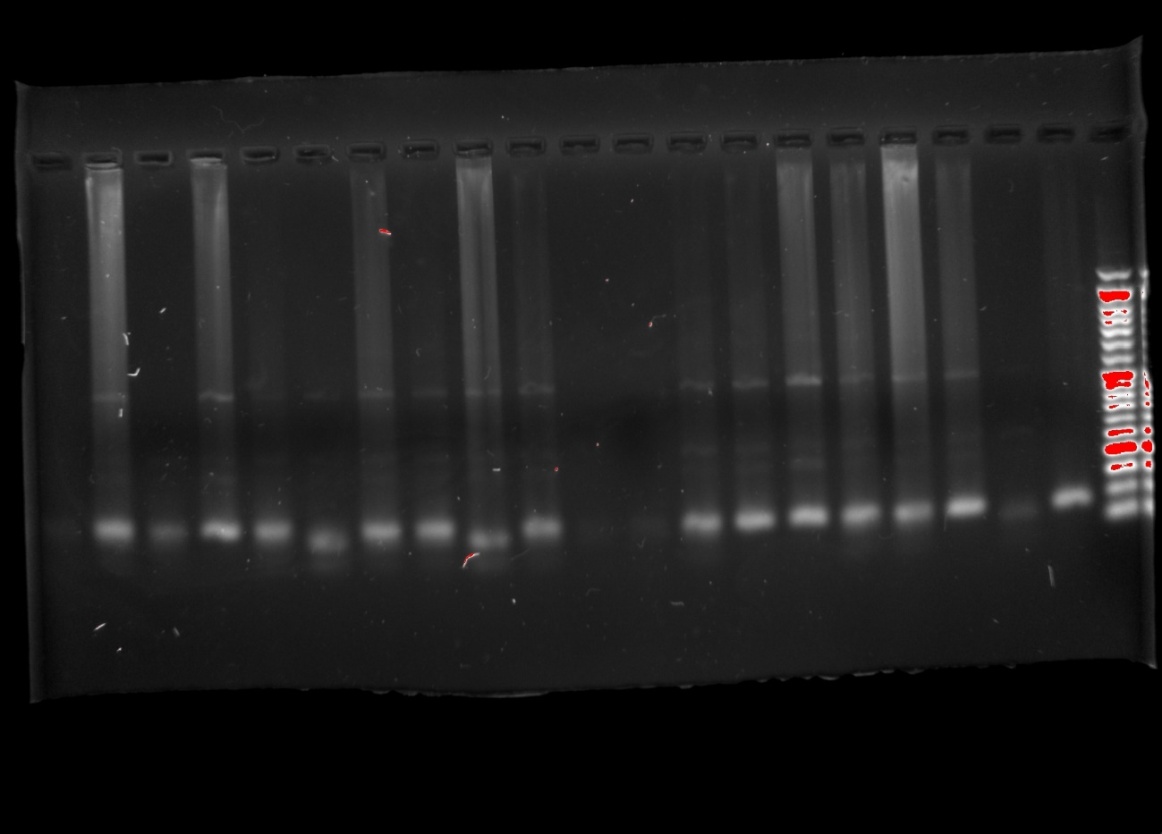
Comparison of IR models among cases and controls showed a significantly low (p<0.0001) HOMA B cell and HOMA 1% B cell (insulin based) as well as significantly high (p<0.0001) HOMA B cell, HOMA 1% B cell (C peptide based) in cases. It was also observed that C peptide based insulin resistance models (HOMA IR -C and CIR) were significantly high (p<0.0001) in cases as compared to cases (fig 2). However there was no significant difference in insulin based HOMA IR and QUICKI, between cases and controls (p=0.604 and p=0.466).

Biochemical parameters were compared among cases with different genotypes of leptin receptor (LepR) gene, AA, AG and GG, no significant difference was observed in Insulin, C peptide, leptin, TG, TC, HDL, LDL and VLDL (p values being 0.22, 0.66, 0.237, 0.65, 0.60, 0.40, 0.62, 0.667 respectively) (fig 3).

IR models, HOMA IR, HOMA 1%B cell, QUICKI, HOMA IRC, HOMA B cell- C, HOMA 1% B cell -C and CIR (both insulin and C peptide based) in different genotypes of LepR didn’t vary significantly (p values being 0.27,0.198,0.185,0.784,0.805,0.59 respectively) (fig 4). However, HOMA B cell significantly varied among LepR genotypes (p<0.0001), values being double in AA genotype, compared to AG (p<0.05), 10 times higher in AA compared to GG (p<0.0001).The value was four times higher in AG compared to GG (p<0.01).

Different genotypic variant of LEPR are represented in fig 8.

Fig 5: Sample Pattern of Distribution of Alleles of Leptin receptor gene (LEPR)



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 (p values being 0.0001, 0.022 and 0.034 respectively) (table5).

Table 5: Comparison of Biochemical parameters in GDM cases with and without IR

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Insulin resistant cases** | **Cases with Normal Insulin sensitivity** | **p value** |
| C-peptide(nmol/L) | 3.15±1.85 | 1.72±1.44 | 0.0001★ |
| Leptin(ng/ml) | 56.31±24.45 | 57.79±23.91 | 0.99 |
| TG(mg/ml) | 285.53±129.76 | 217.26±71.56 | 0.022★ |
| TC (mg/ml) | 216.5±35.26 | 224.31±55.11 | 0.40 |
| HDL (mg/ml) | 50.56±12.01 | 51.8±12.07 | 0.833 |
| LDL (mg/ml) | 137.13±46.85 | 154.19±51.29 | 0.164 |
| VLDL (mg/ml) | 56.66±26.23 | 43.45±14.34 | 0.034★ |

★p value significant

Correlation studies showed a significant negative correlation between FBS and leptin (r=-0.232 p=0.0237). A significant positive correlation was observed between leptin and TG, TC and VLDL levels (r=0.219, p=0.0325, r=0.248, p=0.0150 and r=0.217, p=0.0347 respectively) among GDM patients.

A significant negative correlation was noted between leptin levels and insulin, HOMA IR,HOMA B cell, HOMA 1%B cell and QUICKI among insulin resistant GDM patients (Table 6).

Table 6: Correlation of Leptin levels with IR Models

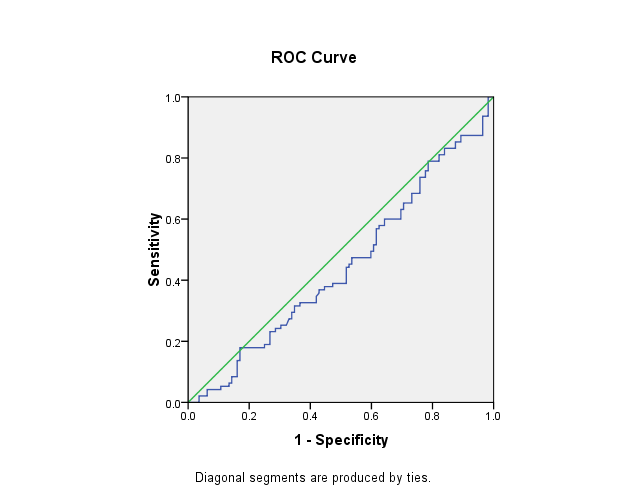
|  |  |  |
| --- | --- | --- |
| **Parameter** | **Spearmann’S correlation ,r** | **p value** |
| Insulin | -0.606 | 0.0005★ |
| C peptide | -0.203 | 0.29 |
| HOMA IR | -0.4856 | 0.0065★ |
| HOMA B cell | -0.4262 | 0.0211★ |
| HOMA 1% B cell | -0.4274 | 0.02★ |
| QUICKI | 0.501 | 0.0056★ |
| HOMA IRC | -0.214 | 0.27 |
| HOMA B cell-C | -0.030 | 0.876 |
| HOMA 1% B cell-C | -0.034 | 0.859 |
| CIR | -0.214 | 0.265 |

★p value significant

ROC was constructed to assess the utility of leptin as a marker of GDM. Area under the curve was 0.446, with a sensitivity of 49.5% and specificity of 60.7% at a cut off value of leptin being 52.7ng/ml (fig 6).

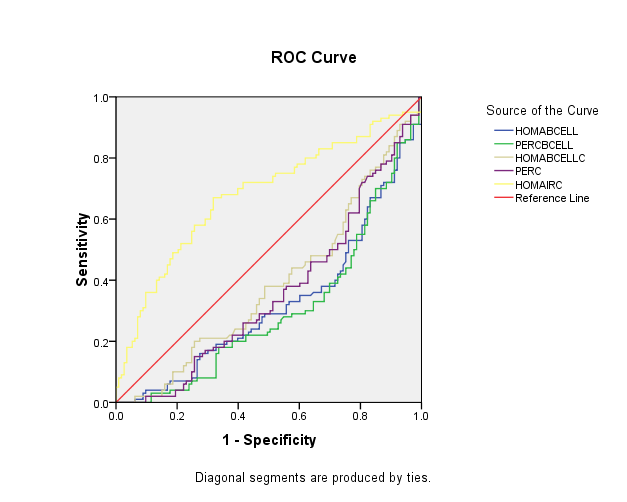
There was no significant association between the birth weight of the babies and different genotypes (p=0.440). The BW of the babies also didn’t differ significantly between the different genotypes (p=0.124). Patients with AA genotypes were 1.5 times at risk of low birth weight babies.

**Fig** **6: ROC for leptin as a marker for GDM**



ROC was also constructed to assess if any of the IR models could be used to predict GDM. It was observed that only HOMA IRC was a better marker with AUC=0.679, with 37% sensitivity and 87% specificity at a cut off value of HOMA IRC being 0.7 (fig 7). However other IR models were found to be poor predictors of GDM.

**Fig 7: ROC of IR models for prediction of GDM**



**Discussion**

No significant association was observed between leptin receptor, (LEPR Gln223Arg) polymorphisms and GDM in our study population. However subjects with homozygous dominant AA allele were at higher risk of (1.25 times) of developing GDM. There was no significant difference between allele distribution between cases and control.

On analyzing *LEPR*Gln223Arg polymorphism, genotype and allele frequencies observed in our study were in accordance to those described in other populations by Boumaiza *et al* [7]. However, lack of significant association between polymorphisms of these genes and GDM could be due to the variation in the genetic patterns in different populations.

The risk of hyperleptinemia was twice among patients with ‘G’ allele as compared to homozygous ‘A’ allele for LEPR gene. An association between *LEPR* Gln223Arg gene polymorphism and leptin levels was found in Thai population studied by Suriyaprom *et al* [8], Dutch population by Rossum *et al* [9] and Romanian subjects by Constantin *et al* [10]. However, no such association was found in Turkish subjects studied by Ornek *et al* [11] and in Chinese population investigated by Yang *et al* [12]. Our results also showed no association between the *LEPR* Gln223Arg polymorphism and other metabolic parameters which are in agreement with the conclusion of Heo *et al.*’s meta-analysis [13]. But other studies suggested that *LEPR* Gln223Arg polymorphism was associated with higher insulin resistance and BMI [7].

No significant difference was observed in the serum leptin levels between GDM patients and subjects with normal glucose tolerance (fig 1). However, hyperleptinemia was observed in both cases and controls, considering 2.5-21.8ng/ml as normal reference range for serum leptin.ROC analysis showed that leptin is not a good marker to predict GDM (fig 6).

In pregnancy, due to the increased fat mass and the presence of placenta, maternal leptin concentrations increase 2 to 3-fold above non-pregnant concentration, with the peak occurring around 28 weeks of gestation[14]. As leptin may have a major role in maternal metabolism and maternal glucose homeostasis regulation, plasma leptin levels may be an important marker for predicting GDM. However, reports available on the levels of maternal leptin in GDM, are conflicting. Studies have reported an elevated leptin levels in GDM [15, 16, 17], diminished leptin levels [3] or insignificant differences in leptin levels among GDM patients compared to controls [18-20]. A study by Noureldeen *et al.* reported no significant change in leptin levels at 2nd trimester but diminished leptin levels at the 3rd trimester among GDM patients [21]. Qiu and colleagues, in their cohort study showed that for each 10 ng/ml increase in the leptin levels in early pregnancy was associated with a 20% increase in GDM risk [22].

Mutation in leptin receptor (LEPR) Gln223Arg results in impaired signaling capacity of leptin receptor [23]. Kautzky-Willer *et al*, in a case control study, found that plasma leptin concentrations were higher in GDM women compared with the control group in the third-trimester [15]. Such a relation was also found in the Vitoratus *et al* [24] and Qiu *et al* [16] studies. Liu *et al* [25] showed that serum leptin level is correlated with glucose tolerance during pregnancy. However, Festa *et al* [3],in a case-control study, noted that maternal third-trimester leptin concentrations were significantly lower in GDM cases as compared with controls after adjusting for possible confounding factors, such as BMI and insulin concentrations. Several possible explanations are suggested for the disparities in the existing studies.

Pregnancy is considered to be leptin resistant state, which is associated with impaired leptin signaling. One possible function of increased maternal leptin levels is to enhance the mobilization of maternal fat stores to increase availability and to support trans-placental transfer of lipid substrates [26]. There is strong evidence that suggest placenta is the main contributor of plasma leptin rather than the adipose tissue [27]. Human placental promoter region might be differently regulated compared to adipose tissue. The fetus may be contributing to the maternal leptin load from early second trimester [28]. A positive correlation was observed between umbilical cord plasma leptin and birth weight of newborns support this finding [29].

Most studies have found increased leptin concentrations in GDM [15, 30, 31, 24]. Moreover, hyperleptinemia in early pregnancy appears to be predictive of an increased risk to develop GDM later in pregnancy independent of maternal adiposity.

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 (fig 3). Comparison of IR models among cases and controls showed a significantly low insulin based IR models, HOMA B cell and HOMA 1% B cell as well as significantly high C peptide based models, HOMA B cell, HOMA 1% B cell 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 (fig2). Since it is an well-established fact that C-peptide is a better marker of endogenous insulin secretion and C-peptide based IR model is a better indicator of insulin resistance, we can conclude that GDM patients have higher IR compared normal glucose tolerant pregnant women.

As HOMA B cell (insulin based) significantly varied among LepR genotypes (p<0.0001), values being double in AA genotype, compared to AG (p<0.05), 10 times higher in AA compared to GG (p<0.0001), four times higher in AG compared to GG(p<0.01), homozygous dominant, AA genotypes are at higher risk of IR and hence GDM. However, ROC analysis showed that none of the IR models were good predictive markers of GDM except for HOMA IRC, with AUC 0.67 (fig 7).

Previous studies have suggested that leptin has an inhibitory effect on insulin gene expression and insulin secretion in pancreatic β-cells. Leptin also suppresses insulin secretion induced by cAMP, glucagon-like peptide 1 and protein kinase C [32, 33]. Moreover, leptin inhibits the phosphorylation of glucose transporter 2 (GLUT2) and impairs glucose transport in tissues increasing insulin resistance

It has been suggested that as gestation progresses, insulin secretion increases, reaching a maximum in the third trimester [34], whereas insulin sensitivity decreases progressively by about 70% [35]. In normal pregnancy, pancreatic beta cells compensate for the increased insulin resistance to control blood glucose [36]. However, in a pregnancy complicated by GDM, reduced insulin secretion capacity and the physiological insulin resistance that occurs on a background of chronic insulin resistance, lead to a deterioration of glucose tolerance. Studies have shown that impaired beta cell function in women with GDM is mainly attributed to decreased early-phase insulin secretion [37]. Moreover, when insulin secretion was adjusted for the degree of insulin resistance, women with GDM had severe reduction in beta-cell function compared to normal pregnant women [37]. Ryan *et al.* demonstrated increased insulin resistance in women with GDM [38]. They reported a decrease in glucose infusion rate during euglycaemic clamp in women with GDM by 40-60% compared to pregnant non-diabetic controls and by 60-70% compared to non-pregnant controls [38]. Furthermore, increased endogenous glucose production has been reported in women with GDM compared to healthy pregnant controls [34, 37]. Leptin directly affects pancreatic β-cell gene expression and leads to decrease insulin secretion [39, 40]. Furthermore, leptin affects the β-cell proliferation, apoptosis, and cell growth [41]. Leptin suppresses the expression of preproinsulin mRNA in pancreatic β-cells. Several signaling pathways are involved in this inhibitory role of leptin in insulin secretion.

Compared with the NGT group, higher leptin levels were found in the IFG group, consistent with the previous study [22, 42]. Similarly, significantly higher fasting insulin levels, HOMA-IR and lower QUICKI were also noted in IFG and IGT group namely impaired fasting glucose individuals, than NGT group. Positive and negative correlations were found between plasma leptin levels and HOMA-IR and QUICKI respectively. These correlations were confirmed in some studies [44, 45], but contradicted in a few studies [46]. The observed positive correlation between plasma leptin concentrations and the maternal pre-pregnant BMI was in accordance with many previous studies both in GDM group and NGT group.

In our study, there was no significant difference between lipid profile parameters like TG, TC, HDL, LDL and VLDL levels between cases and controls (fig 1).On comparing lipid profile among cases with different genotypes leptin receptor genes, AA, AG and GG, no significant difference was observed among them (fig 5). Correlation studies showed a significant negative correlation between FBS and leptin, which supports our finding of hyperleptinemia in GDM patients. 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(Table 6).

Dyslipidemia in pregnancy is well documented. After an initial decrease in the first trimester, there is a steady increase in triacylglycerols, fatty acids, cholesterol, lipoproteins, and phospholipids. The higher concentration of estrogen and insulin resistance is thought to be responsible for the hypertriglyceridemia of pregnancy. Changes in total cholesterol concentration reflect changes in the various lipoprotein fractions. HDL cholesterol increases by 12 wk of gestation in response to estrogen and remains elevated throughout pregnancy [47]. Total and LDL cholesterol concentrations decrease initially, but then increase in the second and third trimesters. VLDL and triacylglycerols decrease in the first 8 wk of gestation and then continuously increase until term. Changes in lipid metabolism promote the accumulation of maternal fat stores in early and mid-pregnancy and enhance fat mobilization in late pregnancy. In early pregnancy, increased estrogen, progesterone, and insulin favor lipid deposition and inhibit lipolysis.

GDM induces a state of dyslipidemia consistent with insulin resistance. During pregnancy, women with GDM do have higher serum triacylglycerol concentrations but lower LDL-cholesterol concentrations than do normal pregnant women [48]. In a study by Nawal *et al*, total cholesterol, HDL cholesterol, and apolipoprotein concentrations were not significantly different between GDM patients and control subjects [49].The results showed that there was positive correlation between leptin with cholesterol, triglyceride, and VLDL in over weight group and in obese group. The negative correlation was found between leptin with HDL in obese group [49].

Leptin synthesis is induced by hyperglycemia, hyperlipidemia, and a replete fat mass and also leptin suppresses insulin production [50]. Leptin is expressed predominantly by adipocyte, which fits the idea that body weight is sensed as a total mass of fat in the body [51]. Serum leptin concentration is increased in obese subjects and is closely related to fat mass and BMI and declines with weight loss [52]. Leptin plays a central role in the long-term maintenance of weight homeostasis by acting on the hypothalamus to decrease food intake and increase energy expenditure [53]. Serum leptin was positively& strongly correlated with BMI which is an important index of obesity. Similar correlation was reported by previous studies [54-56]. Positive correlation was noted between leptin & TG, negative correlation between HDL and leptin [57].

It was also observed in the present study that there was no significant association between leptin receptor gene polymorphisms and insulin resistance. However, Odd’s ratio showed 1.2 times risks of IR in patients with A allele for leptin and leptin receptor 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 (table 3).

Some studies suggest that leptin also has a main effect on the regulation of whole body glucose homeostasis [58, 59]. Some studies demonstrated a positive correlation between direct and indirect measures of adiposity with plasma leptin concentrations [60]. In pregnant women with changes in maternal fat stores and glucose metabolism, leptin increases [14]. Maternal leptin concentration increases 2-3times above the non-pregnant concentration with the peak around 28 weeks of gestation [4]. Studies suggest that increasing maternal plasma leptin may result from an up regulation of adipocyte leptin synthesis in the presence of increasing insulin resistance and hyperinsulinemia in the second half of pregnancy [4]. Researchers have illustrated that leptin directly affects whole body insulin sensitivity through regulating the efficiency of insulin-mediated glucose metabolism by skeletal muscle and by hepatic regulation of gluconeogenesis [61, 62]. The findings of some studies indicate that leptin has an acute inhibitory effect on secretion of insulin. Large epidemiological studies have shown that plasma leptin concentrations were positively associated with insulin resistance in men and non-pregnant women [63].

**Conclusion**

It could be concluded from the study that, there is no significant association between leptin receptor, LEPR Gln223Arg alleles and gestational diabetes, leptin levels and insulin resistance. However, subjects with ‘G’ allele for LEPR at higher risk of hyperleptinemia. C –peptide based insulin resistance models were elevated in GDM patients. Subjects with homozygous dominant, ‘AA’ of LEPR showed higher insulin resistance, HOMA B cell compared to other genotypes. A positive correlation was seen between leptin levels and triglycerides, total cholesterol and VLDL levels in GDM.TG and VLDL were significantly higher in IR resistant GDM patients. Leptin showed a significant negative correlation with insulin levels, HOMA-IR and positive correlation with QUICKI in IR cases. The study is able to establish a cycle of gene polymorphism altering leptin levels which in turn can alter insulin secretion and insulin resistance, contributing for dyslipidemia of pregnancy as well as gestational diabetes.

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**Conflicts of interest:** None

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