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A Cross-Sectional Study On The Association Of Leptin Gene Polymorphism And Metabolic Parameters In Gestational Diabetes Mellitus

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Abstract

Background: Gestational diabetes mellitus (GDM) is a pregnancy condition marked by poor glucose tolerance caused by insulin resistance. Objective was to find association between leptin gene with leptin levels, insulin resistance as well as lipid profile in patients with GDM as compared to pregnant women with normal glucose tolerance.

Methods: 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. Insulin resistance models were calculated using 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 calculated to study the extent ofrisk of leptin gene polymorphism in causing GDM. 'p'<0.05 was regarded as statistically significant.

Results: There was no association seen between the leptin gene polymorphism and GDM, leptin levels, or insulin resistance. In cases, IR models revealed significantly lower ($p<0.0001$) HOMA B cell and HOMA 1 percent B cell (insulin based) and significant elevation ($p<0.0001$) of the same. C-peptide-based insulin resistance models were also found to be considerably higher ($p<0.0001$) in cases compared to controls.

Conclusion: There is no evidence that LEPG2548A alleles are linked to GDM, leptin levels, or insulin resistance. Insulin resistance models based on C–peptide were found to be higher in GDM patients.

Keywords: Leptin, Gene polymorphism, Leptin gene, Insulin resistance, Gestational Diabetes.

I. INTRODUCTION

Gestational diabetes mellitus (GDM) is characterized by glucose intolerance of various severity that develops during pregnancy or is first identified during pregnancy [1]. GDM affects about 7% of all pregnancies around the world. However, Choudhary *et al.* reported a prevalence of 9% in India in a recent research [2]. GDM is caused by a loss in insulin sensitivity, which leads to altered metabolic consequences. Leptin levels have been observed to be altered in GDM [3, 4]. They may be elevated or decreased. The existing reports are contradictory, and the facts have yet to be proven. Elevated leptin levels have been linked

to insulin resistance in GDM [5]. Because leptin is linked to metabolism of lipids, it may play a role in dyslipidemia in gestational diabetes.

Gene polymorphisms in GDM:

Several investigations have found that gene polymorphisms play a role in the development of GDM. Hofstadter and colleagues found a link between the polymorphism of LEP G2548A and raised leptin levels in their study [6]. Yang *et al* investigated the polymorphisms of the leptin gene and its receptor in the Chinese population. In the Indian population, there are no similar reports. According to clinical trial findings, elevated leptin levels are caused by upregulation of the leptin gene as a result of insulin resistance and hyperinsulinemia [5]. Leptin has been shown to impact insulin sensitivity by regulating glucose metabolism via insulin in muscle and hepatic gluconeogenesis regulation [7]. Insulin secretion was discovered to be inhibited by leptin [8].

Elevated leptin levels were linked to a high TG/HDL-C ratio in a study by Lekva*et al* [9]. GDM patients' lipid levels may be affected by altered leptin levels and insulin resistance. Insulin resistance is a critical element in the progression of GDM. This condition is caused by a combination of reduced maternal pregravidinsulin sensitivity and insufficient response to insulin.

Objectives of the study were to:

1. Assess the pattern of polymorphism of leptin gene (LEPG2548A) in GDM and to find the association of serum leptin levels with it
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.

II. METHODS

Study design &Setting: This cross-sectional study was carried out from July 2018 - July 2020 and it was conducted in Endocrinology and molecular genetics wing ofCentral Research Laboratory of K.S. Hegde Medical Academy, India.

This study follows 'The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement guidelines.A completed STROBE checklist can be found in the Reporting guidelines. The study flow as per STROBE guidelines is depicted in the Figure 1.

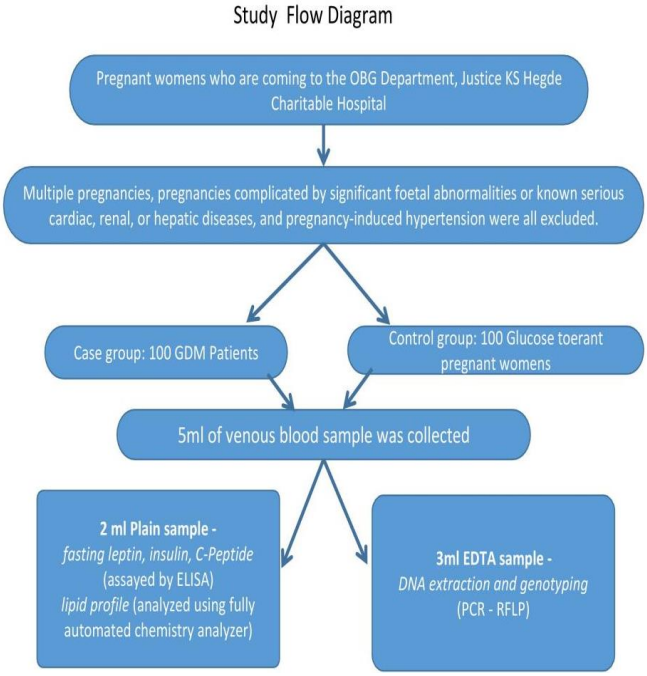

Figure1: *Study Flow Diagram*

III.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. As a control group, 100 normal glucose tolerant pregnant women of similar gestational age and BMI were recruited.

Exclusion Criteria for cases and control:

Multiple pregnancies, pregnancies complicated by significant foetal abnormalities or known serious cardiac, renal, or hepatic diseases, and pregnancy-induced hypertension were all excluded.

To assess if the patient meets pre-determined criteria, a proforma covering general information on demographic parameters, parity, family history of diabetes and hypertension, and past history of GDM was acquired. Before beginning the study, the NU Central Ethics Committee gave their consent. Patients provided written informed consent.

Collection and analysis of blood sample: Five milliliters of venous blood samples were drawn from the patients. Two ml of blood was collected in plain vial was utilized for fasting leptin, insulin, C-Peptide (assayed by ELISA) and lipid profile (analyzed using fully automated chemistry analyzer). Insulin resistance models (both insulin and C-peptide based) were constructed using appropriate formulae (Table 1).

Table 1:*Formulae for calculating insulin resistance models*

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

Genetic analysis: Three ml of venous blood sample, collected in EDTA (2%) vial was utilized for DNA extraction and genotyping. 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. Details of primers and restriction enzyme used are depicted in Table 2.

Table 2: *Details of PCR-RFLP for the gene Leptin*

SNP	**Location of the allele (Base change)**	**Forward and Reverse Primers**	**PCR Program (35 cycles)**	**PCR Fragment length (Bp)**	**Restriction enzyme, Incubation temperature**	**Allele: RFLP fragment size**
LEP (rs7799039)	Promoter (G>A)	F5'-TTTCCTGTAATTTTCCCGTGAG-3' R5'AAAGCAAAGACAGG CATAAAAA-3'	93°C,45', 61°C,30', 72°C,30'	242	HhaI, 37°C	Allele A:242 Allele G:181+61

Sample size calculation

There are not many such studies in the literature so far estimating the correlation of leptin and leptin gene polymorphism in the Indian population. However, by extracting the information derived from the studies published so far on LEP G2548A and 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

SPSS 23.0 software was used to carry out the statistical analysis. Hardy-Weinberg Equilibrium (HWE) was performed among cases for the LEP gene variant, and the chi-square test was used to analyse the distribution of allele frequencies between variants. For associations, the Chi-square test was performed, Mann Whitney U to compare biochemical parameters between cases and controls, Spearman's correlation test for correlations. The chance of a leptin gene polymorphism causing GDM was calculated using Odd's ratio. A 'p' <0.05 was considered as statistically significant.

IV. RESULTS:

The patient characteristics (Age, body mass index and gestational age were given in Table 4 indicating that the groups are comparable. The mean age of cases and controls were 29.62±4.3 yrs and 27.08 ±3.73 yrs . Mean body mass index of the groups were 25.78 ± 6.84 kg/m^2 and 25.86 ± 5.86kg/m^2among cases and controls respectively. Gestational age of the subjects was 25.87± 1.21 weeks and 26.1±1.54 weeks respectively.

Leptin gene polymorphism pattern: The frequencies of genotypes and alleles of LEP (rs7799039) in Table 3 & Figure 1.

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

Gene variant		Frequency genotypes LEP gene (rs7799039)		Chi -square
		Cases	Controls	
AA	Observed	28	27	0.47 (Cases) 0.654 (Control)
	Expected	29.7	24.8	
AG	Observed	53	52	
	Expected	49.6	56.2	
GG	Observed	19	34	
	Expected	20.7	31.8	
Association between GDM and LEP Gene polymorphism (Chi-square)				0.1694 p=0.68

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

In GDM cases, none of the genotype frequency distributions for rs7799039 variations varied significantly from HWE (P>0.05), implying that alleles were in equilibrium. Association studies between 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 a no significant association between them (Table 3). Individuals with the A allele, on the other hand, had a 1.25 times higher risk of getting GDM, as determined by Odd's ratio.

There was no evidence of a link between LEP gene variations and leptin levels with Chi-square statistic and Yates correction being 0.0626 (p=0.802). There was also no significant link found between leptin gene polymorphisms and insulin resistance (Chi-square statistic = 0.805, p=0.369). Subjects with homozygous dominant AA alleles, on the other hand, had a 1.25-fold

increased chance of developing GDM. Patients with the 'A' allele for the leptin gene had 1.4 times the risk of IR, according to the odds ratio.

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). In GDM patients, fasting serum insulin and leptin levels were insignificantly low (p=0.6968 and p=0.213, respectively). Lipid profile measurements such as TG, TC, HDL, LDL, and VLDL showed no significant differences(pvaluesbeing0.343,0.091,0.57,0.61, 0.65,0.097,0.157,0.12 respectively) between cases and controls as showed in Table 4.

Table 4:*Depicting metabolic parameters in cases and controls*

Parameter	GDM	Control	p value	Spearman's Correlation	
				r value	p value
Mean age(yrs)	29.62±4.3	27.08 ±3.73			
BMI (kg/m$^{2)}$	25.78 ±6.84	25.86±5.86			
Gestational age(wk)	25.87± 1.21	26.1±1.54			
FBS(mmol/L)	7.49±1.87	4.95±1.32	<0.0001*		
Fasting Insulin μIU/L	5.46±11.95	7.13±6.74	0.6968	-0.606	0.0005*
C-peptide(nmol/L)	2.17±1.71	1.57±1.55	0.0014	-0.203	0.29
Leptin(ng/ml)	57.33±23.96	63.11±25.46	0.213	-	-
TG(mmol/L)	2.68±1.1	2.79±0.83	0.069	-	-
TC (mmol/L)	5.75±1.29	5.99±1.19	0.12	-	-
HDL (mmol/Ll)	1.33±0.31	1.41±0.32	0.73	-	-
LDL (mmol/L)	3.85±1.3	4±1.16	0.255	-	-
VLDL (mg/dl)	1.23±0.51	1.26±0.35	0.06	-	-
HOMA IR	2.94±4.64	1.63±2.09	0.604	-0.4856	0.0065*
HOMA B cell	35.78±50.55	75.73±90.89	<0.0001*	-0.4262	0.0211*
HOMA 1% B cell	42.62±63.75	114.03±156.99	<0.0001*	-0.4274	0.02*
QUICKI	2.39±3.46	8.89±9.8	0.466	0.501	0.0056*
HOMA IRC	0.7±0.62	0.36±0.38	<0.0001*	-0.214	0.27
HOMA B cell-C	11.03±10.07	17.31±17.25	0.0045*	-0.030	0.876
HOMA 1% B cell-C	13.58±13.16	24.65±44.5	0.0002*	-0.034	0.859
CIR	3.13±5.07	11.47±31.17	<0.0001*	-0.214	0.265

*p value significant

In cases, there were considerably lower (p<0.0001) HOMA B cell and HOMA 1 percent B cell (insulin based) as well as significantly higher (p<0.0001) HOMA B cell and HOMA 1 percent B cell (C peptide based) IR models than in controls (table 4). In addition, C peptide-based insulin resistance models (HOMA IR -C and CIR) were found to be considerably higher (p<0.0001) in cases than in controls (Table 4). Table 4 shows that there was no significant difference between patients and controls in insulin-based HOMA IR and QUICKI.

Biochemical parameters and Insulin resistance with Leptin gene polymorphism:

On comparing biochemical markers among subjects with different leptin genotypes, AA, AG and GG, Insulin, C peptide, leptin, TG, TC, HDL, LDL, and VLDL levels did not differ significantly (p= 0.343, 0.091, 0.57, 0.61, 0.65, 0.097, 0.157, 0.12) (Table 5).

Table 5:*Depicting metabolic parameters in cases with different genotypes of leptin gene*

	AA	AG	GG	p-value
FBS (mmol/L)	7.76 ± 1.70	7.71 ± 1.70	6.39 ± 2.36	0.030*
Insulin (µIU/L)	10.16 ± 14.76	9.11 ± 12.05	4.17 ± 4.74	0.343
C-peptide (nmol/L)	1.91 ± 1.26	2.53 ± 1.94	1.58 ± 1.42	0.091
Leptin (ng/mL)	56.3 (14.34-102.6)	56.8 (7.7-98.03)	69.4 (2.7-92.88)	0.579
Triglyceride (mmol/L)	2.32 (1.1-4.65)	2.5 (0.99-6.21)	2.32 (1.77-2.88)	0.618
Cholesterol (mmol/L)	5.97 (2.9-8.07)	5.69 (3.1-9.78)	5.61 (4.01-8.28)	0.659
HDL (mmol/L)	1.69 (0.75-2.02)	1.32 (0.78-2.04)	1.27 (0.72-1.71)	0.098
LDL (mmol/L)	3.92 (1.66-5.87)	3.55 (1.22-7.76)	4.32 (2.04-6.15)	0.157
VLDL (mmol/L)	1.05 (0.49-2.12)	1.14 (0.47-3)	1.06 (0.8-1.53)	0.121

Different insulin resistance 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 various leptin genotypes didn't vary significantly (p values being 0.402, 0.946, 0.912, 0.99, 0.074, 0.32, 0.17, 0.07 respectively) (Table 6).

Table 6: *Comparison of IR models in different genotypes of Leptin gene*

	AA	AG	GG	p-value
HOMA-IR	3.57 ± 1.14	3.25 ± 0.95	1.15 ± 0.34	0.402
Glucose/Insulin	10.7 ± 1.91	19.8 ± 2.27	31.8 ± 2.95	0.674
HOMA -B Cell	35.4 ± 15.27	35.3 ± 15.89	37.7 ± 17.05	0.946
HOMA 1% - B Cell	39.4 ± 16.4	40.8 ± 19.2	53.5 ± 18.7	0.912
QUICKI	1.023 ± 0.97	4.87 ± 0.96	4.98 ± 0.91	0.992
HOMA IR C	0.58 ± 0.38	0.85 ± 0.67	0.44 ± 0.37	0.074
HOMA B Cell C	10.17 ± 5.36	10.98 ± 6.94	12.42 ± 9.15	0.322
HOMA 1% B Cell C	12.6 ± 5.97	13.04 ± 9.99	16.57 ± 3.08	0.17
CIR	3.12 ± 1.34	3.56± 1.78	4.14 ± 1.89	0.07

Discussion:

The frequency of A alleles was found to be higher than G alleles for LEP G2548A polymorphism (Table 3). Similar pattern of 'A' allele predominance was seen in Taiwanese study by Wang *et al* [10]. However, research from many populations have found that the G allele is more common than the Aallele [11-13]. Only a few investigations have explored the association between the LEP G2548A polymorphism and GDM. Vasku*et al.* found that GDM patients with the AA and AG genotypes have a considerably higher risk of GDM than those with the GG genotype in a Czech study with a lower sample size [11]. The current investigation found no link between the LEP G2548A polymorphism and GDM (Table 3).

Gene polymorphism and Leptin levels*:*

This study also found no link between polymorphisms in the leptin gene and its expression in the blood. Previous reports have found that the LEP G2548A polymorphism has a considerable impact on leptin gene expression [14,15], which contradicts the findings of an Egyptian study by Abdel [16] and a Romanian study by Constant in *et al* [3]. According to Vasku*et al*, people with the mutant variant (AA) and heterozygous (AG) alleles had a significantly higher risk of gestational diabetes mellitus than those with the GG genotype due to the higher transcriptional activity of the LEP gene [11]. This study backs up the theory that leptin plays a role in the etiopathogenesis of GDM.

Pawlik*et al.*, Sahin*et al.*, Mammès*et al.*, and Hoffstedt*et al.* found a link between the LEP rs2167270 A allele and leptin overexpression and enhanced transcriptional activity [6, 17-19]. When leptin levels were evaluated among GDM patients with different genotypes, patients with the AA allele had the highest serum leptin levels, despite the fact that the differences were negligible. Maternal serum concentration of leptin rise two to three times over non-pregnant levels during pregnancy, with a peak value being noted at 28 weeks of pregnancy [20]. However, there are inconsistent data on maternal leptin levels in GDM. Studies have found that GDM patients have higher leptin levels, lower leptin levels, or no significant variations in leptin levels when compared to controls [3, 4, 21-24].

In a study of GDM patients, Noureldeen*et al.* found no significant alterations in leptin concentrations in the second trimester, whereas decreased leptin levels in the third trimester [3]. In their cohort analysis, Qiu and colleagues found that each 10ng/ml rise in leptin levels during initial trimesters of pregnancy was related with a 20% higher risk of GDM [25].

In comparison to healthy pregnant women, serum leptin concentrations and placental expression of leptin were considerably higher in GDM [26, 27]. As a result, mutations in the leptin and its receptor genes may affect the expression of their proteins. In a case-control study, Kautzky-Willer *et al* discovered that in the third trimester, leptin levels were greater in GDM women than in the control group [28].

Research by Vitoratus*et al* [29] and Qiu*et al* [25] also discovered an association of the leptin gene and its expression. The level of blood leptin is linked to glucose tolerance during pregnancy, according to Liu *et al* [30]. Festa *et al* [4] found that in GDM cases, maternal third-trimester leptin concentrations were considerably lower. Elevated maternal leptin concentration may improve the mobilization of maternal stored adiposity, hence supports trans-placental lipid substrate transfer [31]. There is significant evidence that the placenta is the primary source of plasma leptin [32]. In comparison to adipose tissue, the human placental promoter region may be regulated differently. It's possible that the foetus is adding to the mother's leptin loadfrom early second trimester [33]. This conclusion is supported by a positive correlation between umbilical cord plasma leptin levels and birth weight of the babies [34].

Increased leptin concentrations have been identified in GDM in the majority of studies [28, 35, 36, 29, 30]. Furthermore, regardless of maternal adiposity, hyperleptinemia in early pregnancy appears to predict an increased risk of developing GDM later in pregnancy.

In our study, no significant difference was observed in serum insulin concentrations of GDM cases and controls, but C-peptide was higher significantly (p=0.0014) among cases (Table 4). On comparing among cases with different genotypes of leptin gene, AA, AG and GG, Insulin and C peptide concentrations were not significantly altered (p=0.343 p=0.091).

Polymorphisms of leptin gene could result in altered expression of their proteins. However, in the present 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 (table 4). There was no significant difference in C-peptide levels among various genotypes.

Insulin resistance and leptin polymorphism: Comparison of insulin resistance models showed a significantly low insulin based IR models (HOMA B cell and HOMA 1% B

cell)($p<0.0001$) as well as significantly high C peptide based IR models (HOMA B cell C,HOMA 1% B cell C)($p<0.0001$) 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 (table 4). Glucagon-like peptide 1, cAMP and protein kinase C all cause insulin production to be suppressed by leptin [37, 38]. Insulin secretion is thought to rise as pregnancy advances, peaking in the third trimester [39], whereas insulin sensitivity declines by roughly 70% [40]. Beta cells of pancreas compensate for heightened insulin resistance in normal pregnancy to maintain blood glucose control [41]. Reduced early-phase insulin production has been linked to reduced beta cell activity in women with GDM, according to studies [42]. Furthermore, when insulin secretion was corrected for insulin resistance, women with GDM showed significantly worse beta-cell function than normal pregnant women [64]. In women with GDM, Ryan *et al.* found greater insulin resistance [43]. Furthermore, when compared to healthy pregnant controls, women with GDM had higher endogenous glucose production [39, 42]. Leptin has a direct effect on pancreatic-cell gene expression, resulting in a reduction in insulin release [44, 45]. Leptin also influences -cell proliferation, apoptosis, and cell growth [46]. The IFG group had higher leptin levels than the NGT group, which was consistent with a prior study [24]. Plasma leptin levels were found to have positive and negative relationships with HOMA-IR and QUICKI, respectively. Some research [26, 27] corroborated these associations, while others [47] refuted them.

Lipid parameters and gene leptin polymorphism: There was no significant difference in lipid profile markers such as TG, TC, HDL, LDL, and VLDL levels between patients and controls in our study (table 4).

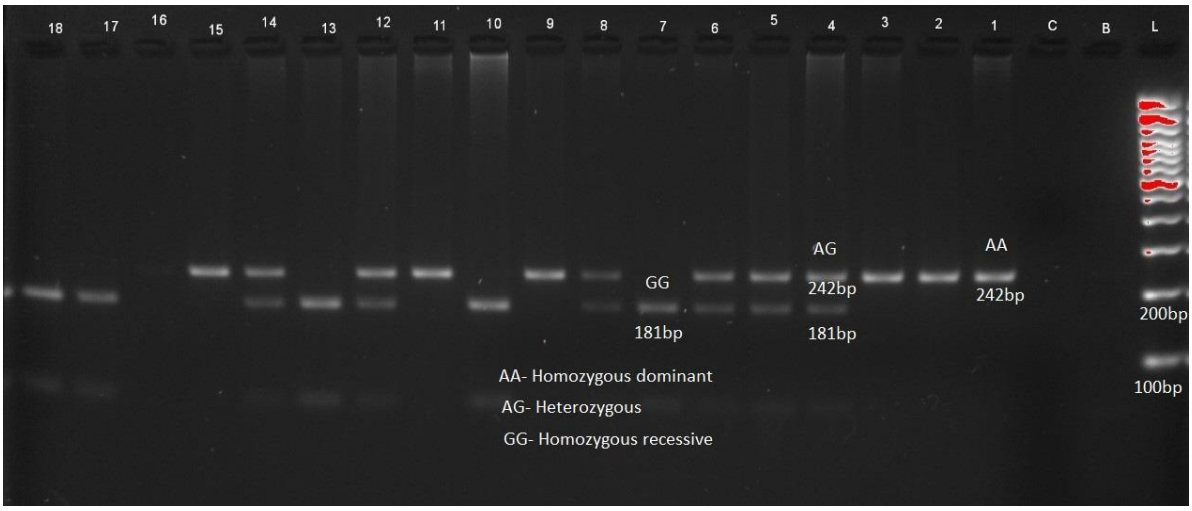

Figure 1:*RFLP Pattern for the distributionof LEP gene alleles*

FBS and leptin had a substantial negative association in correlation studies. In GDM patients, there was a substantial positive connection between leptin and TG, TC, and VLDL levels. Among insulin resistant GDM patients, there was a substantial negative connection between leptin levels and insulin, insulin-based IR models, HOMA IR, HOMA B cell, HOMA 1 percent B cell, and QUICKI. (Table 4).

Pregnancy-related dyslipidemia is well-known fact. In a response to oestrogen, HDL cholesterol rises at 12 weeks of pregnancy and remains raised throughout the pregnancy [48]. Women with GDM have greater blood triacylglycerol concentrations than normal pregnant women, but lower LDL-cholesterol values [49]. In a research study by Nawal *et al*, between GDM patients and control subjects, total cholesterol, HDL cholesterol, and apolipoprotein levels were not significantly different [50].

Limitations of the study: Small sample size was limitation for this study.

Generisability: the study may be carried out in a larger population along with measurement of leptin resistance.

V. CONCLUSION

According to our study findings, there is no association between the LEPG2548A allele and gestational diabetes, leptin levels, or insulin resistance. In GDM patients, C-peptide-based insulin resistance were raised. The findings could lead to a cycle in which a leptin gene polymorphism affects leptin levels, which then affects insulin secretion and resistance, contributing to pregnancy induced dyslipidemia and gestational diabetes.

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Competing interests:

None

Data availability

Figshare. A Cross-sectional Study on The Association of Leptin Gene Polymorphism And Metabolic Parameters in Gestational Diabetes Mellitus. DOI: https://doi.org/10.6084/m9.figshare.20321739.v1

This project contains the following underlying data:

- Leptin CONTROL and CASE Dataset
- Study flow diagram

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