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Gestational Diabetes Mellitus And Leptin Gene polymorphism In Egyptian Women

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Abstract

Gestational diabetes is blood sugar elevation during pregnancy and exposes women to the risk for development of type 2 diabetes mellitus in later years of life. The LEP rs7799039 (2548G/A) polymorphism in the 5´ region of the LEP gene was reported not only to be associated with overweight but also to have a strong influence on leptin gene expression and adipose tissue secretion. The aim of this study is to evaluate the distribution of LEP rs7799039 (2548G/A) polymorphism and its plasma level in patients with gestational Diabetes Mellitus. This study was carried out on160 subjects divided into 2 groups; 80 patients with GDM (group I) and 80 healthy subjects served as controls (group II). All studied subjects were submitted to full history taking, general clinical examination and laboratory investigations including fasting and 2 hour post prandial blood glucose, total cholesterol (TC),triglycerides (TG), HDLc, LDLc, glycated hemoglobin (HbA1c), plasma leptin, fasting serum insulin, HOMA- IR and Genotyping of LEP rs 7799039 (2548G/A) polymorphism was analyzed by the TaqMan allelic Discrimination Assay Technique. Results showed significant statistical differences between the two studied groups regarding family history and BMI. Significant statistical difference between group (I) and group (II) regarding plasma leptin level (P value <0.001), serum insulin (P value <0.001), insulin resistance (P value <0.001) and LEP rs 7799039 (2548G/A) genotype distribution with increased frequency of the AG and AA genotype in patients with gestational diabetes (P value <0.001) and increased frequency of GG genotype in controls. **Conclusion:** This results indicate that leptin plays a role in increasing insulin sensitivity, LEP rs7799039 (2548G/A) and its plasma level can contribute to susceptibility to GDM and might be used for screening of the early detection of GDM.

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Introduction

Gestational diabetes mellitus (GDM) is one of the most common medical complications of pregnancy (1) and is defined as any degree of glucose intolerance with the first recognition during pregnancy. This includes undiagnosed diabetes mellitus detected for the first time during pregnancy(2).Gestational Diabetes Mellitus and gestational dysregulation of blood glucose levels expose the women affected to a higher risk for subsequent development of type 2 diabetes mellitus and cardiovascular disease later in their lives(3,4).

The adverse outcomes associated with GDM for both the mother and the offspring are diverse. They include short-term complications such as macrosomia, neonatal hypoglycemia, preeclampsia, as well as long-term risk of type 2 diabetes mellitus, obesity and cardiovascular diseases both in the mother and in their offspring(5). The risk of GDM women is increased by advanced maternal age, ancestral disparities, obesity, and a family history of diabetes; however, the exact etiology is unknown due to limited knowledge of genetic factors (6).

Leptin affects the balance of cytokines in the feto-placental unit (7), and therefore the pregnancy outcome (8,9). Also, leptin synthesized in the placenta acts through binding to leptin receptors, which are expressed in the trophoblast (10, 11). Secretion of leptin influence body weight, positively associated with percentage body fat, suggesting that leptin levels is mediating adiposity signals to the brain (12). Excessive production of leptin is a consequence of resistance to its effect on target organs (13), and increased levels are associated with high BMI and insulin resistance in type 2 diabetes patients (T2DM) (14).

One of the mechanisms underlying glucose metabolism in pregnancy are a group of substances, produced mainly in the adipose tissue which includes leptin and adiponectin (15).The adipokines; adiponectin and leptin have been shown to play a role in normal pregnancy, as well as in complications of pregnancy, including GDM(16). The LEPG2548A SNP (rs7799039) is a G → A transition at nucleotide position -2548 upstream of the ATG start site in the LEP gene 5′ promoter region (17).The leptin (LEP) G2548A polymorphism has been associated with increased leptin production and plasma secretion from adipocytes(18).

Subjects and methods

Subjects:

This case–control study included (160) subjects: (80) GDM and (80) healthy, age- and sex-matched subjects as a control group. Cases were selected from Outpatient Clinic of Obstetrics and Gynecology Department, Menoufia University Hospital, Egypt. All studied subjects were subjected to complete history taking, physical examination including anthropometric measurements. Determination of gestational age by the last menstrual period and confirmed by ultrasound. Estimation of pre pregnancy body mass index [BMI] was done by dividing body weight in kilograms by (height in meter2) (19). Gestational diabetes mellitus was diagnosed at any time in pregnancy according to the 2013 WHO (20) criteria for diabetes if one or more of the

following criteria are met; fasting plasma glucose 5.1-6.9 mmol/l (92 -125 mg/dl) or 1-hour plasma glucose ≥ 10.0 mmol/l (180 mg/dl) following a 75g oral glucose load or 2-hour plasma glucose 8.5-11.0 mmol/l (153 -199 mg/dl) following a 75g oral glucose load.

Laboratory investigations including lipid profile [serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDLc) and low density lipoprotein cholesterol (LDLc)].fasting blood glucose, 2 hour post prandial glucose, glycated hemoglobin (HbA1c), fasting serum insulin, plasma leptin by ELISA assessment of insulin resistance by homeostatic model assessment (HOMA)**(21)** and genotyping of *leptin* (*LEP*) G2548A using the TaqMan allelic Discrimination assay technique (real time PCR).

Sample collection and assay

Prior to the collection of samples, written consent (approved by the Committee of Human Rights in Research at Menoufia University) were obtained from all studied cases and control subjects.

After 12 hours overnight fasting, 12 ml of venous blood were withdrawn from every subject by sterile vein-puncture and divided into three tubes; 4 ml of blood were transferred into two EDTA tubes: one of them was used for quantitative colorimetric determination of glycated hemoglobin using kits supplied by Teco diagnostics, USA**(22)** and the other EDTA tube for genotyping of leptin gene, One ml of blood was transferred into sodium fluoride tube for enzymatic colorimetric determination of blood glucose using Spinreact kit, SPAIN **(23)** and 5 ml into tubes without additive, left 10 minutes for coagulation, then centrifuged at 3000 rpm for 10 minutes then sera were used for colorimetric determination of serum TC(24),HDL(25),LDL(26),TG(27) and fasting serum insulin level (28). 2 ml of blood were transferred into EDTA containing tube, centrifuged for 10 minutes at 4000 r.p.m. The clear supernatant plasma was kept frozen at -80° C until determination of plasma leptin level by enzyme linked immunosorbent assay method using DRG® leptin ELISA kit , GERMANY with a detection range (7.36 ± 3.73) in Females (29).

Serum insulin was determined by enzyme linked immunosorbent assay method using DRG® Insulin ELISA kit ,GERMANY(28). Assessment of insulin resistance was done by homeostatic model assessment (HOMA) according to (30). HOMA-IR = fasting glucose (mg/dl) x fasting insulin (μIU/mL) / constant (405).

Genotyping of leptin G2548A (rs7799039) polymorphism:

DNA was extracted from blood samples using GeneJET Whole Blood Genomic DNA Purification Mini extraction Kit, Thermo FisherScientific, USA. DNA was eluted and stored at -20 ^{C} for further PCR procedure.

Leptin Receptor gene was genotyped using allelic discrimination assay by real time PCR technique using TaqMan probe, AppliedBiosystems,USA. The maxima probe qPCR Master Mix (40X),primers and probes were supplied from Thermo Fisher Scientific; the forward primer was 5´-TTTCCTGTAATTTTCCCGTGAG and the reverse primer was 5´-AAAAGCAAAGACAGGCATAAA.10 µl of master mix was added to 1.25 µl of the genotyping

assay of primer/ probe mix and 3.75 µl of DNAase-free water. 5 µl of genomic DNA extract for every sample and 5 µl of DNAase-free water for the negative control reaction were applied.. The following cycling conditions were adjusted: Initial denaturation was done at 95°C for 10 minutes, followed by 40 cycles of: denaturation at 94°C for 15 seconds, primer annealing at 50°C for 60 seconds then extension at 72°C for 2 minutes and the last extension at 72°C for 1 minute. Analysis of data was accomplished using 7500 Real-Time PCR instrument, version 2.0.1, Applied Bio systems.

Statistical Analysis

Results were collected, tabulated and statistically analyzed by IBM personal computer and statistical package SPSS version 20. Hardy-Weinberg equilibrium was computed to exclude any bias of results. Student t-test used for comparison between two groups having quantitative variables. Chi square test ($\chi 2$): was used to study association between two qualitative variables. Mann Whitney and Kruskal–Wallis tests for comparison two and three groups of not normally distributed variables respectively. P-value < 0.05 was considered statistically significant.

Results

The study was conducted on a total number of 160 subjects divided into two groups as follows; 80 patient with gestational diabetes as group I and 80 healthy persons as group II. There was a statistically significant difference between the two studied groups regarding; age, serum FBG,HBA1C, TC,TG ,LDLc, HDLc ,SBP, DBP,2HPP, fasting Insulin, HOMA-IR, baby WT, plasma leptin ,family history of DM and pre pregnancy BMI (P <0.001), while there was no significant difference as regards gestational age (Table 1).

As regards Leprs7799039 genotype distribution between the two studied groups showed a significant difference, with increased frequency of the AA and AG genotypes and A allele in the patient group and increased GG genotype and G allele frequency in the control group (P <0.001; Table 2 and Figure 1,2). The results also showed that the AA genotype of Lep rs7799039(G/A) increases the risk of GDM by 8.3- fold and AG genotype increases the risk by 4.1-fold, while the A allele increases the risk by 4.2-fold, as shown in Table 2. In group I, we compared the three different genotypes of of Lep rs7799039(G/A) (GG, AG and AA) regarding serum FBG,HBA1C, TC,TG ,LDLc, HDLc, SBP, DBP,2HPP, fasting Insulin, HOMA-IR, baby WT, plasma leptin and BMI.

There were significant statistical differences found between the three genotypes regarding these parameters with the highest plasma leptin in AA genotype as shown in (figure 5 and Table 3)..Multivariate logistic regression for risk of GDM showed that the most common risk factor is plasma leptin OR; 32.1(6.1-169.5), followed by Birth weight OR; 22.7 (6.5-79), HOMA-IROR; 9.2(3.01-28.3),AA genotype OR; 9.06(2.06-10.7) Table 4.

There was a significant positive correlation (r=-0.9 and P<0.001) between plasma leptin levels and insulin resistance represented by HOMA-IR in group I. Also there was a significant positive

Table (1): Demographic and clinical characteristics in GDM (group1) and control (group2)

	group I N=80	Group2 N=80	T test	P value
Age (years)	27.5±4.2	24.8±4.9	3.7	<0.001
Gestational age (weeks)	35.7±3.2	35.8±3.7	0.251	0.802
Pre pregnancy BMI (Kg/m^2)	27.4±2.6	21.3±2	16.8	<0.001
SBP (mmHg)	140.4±12.1	111.6±9.5	16.7	<0.001
DBP(mmHg)	92.1±7.9	77±7.7	12.3	<0.001
FBS(mg/dl)	215.6±32.8	89.1±8.3	33.5	<0.001
2HPP(mg/dl)	244.5±33.5	88.9±9	40.1	<0.001
HBA1$_C$%	7.7±1	5.2±0.9	17.8	<0.001
HDL-C(mg/dl)	31.9±1.5	48.5±1.2	77.1	<0.001
Total Cholesterol(mg/dl)	212.4±27.8	170.6±9.9	12.7	<0.001
LDL-C (mg/dl)	147.7±27.7	103.4±9.2	13.6	<0.001
F.Insulin (uIU/ml)	20.8±2.7	4±0.4	54	<0.001
HOMA-IR	11.1±2.7	0.9±0.1	33.4	<0.001
Baby WT (Kg)	4.1±0.4	3.1±0.3	18	<0.001
Plasma leptin(ng/ml)	41.2±7.6	11.2±3.2	32.7	<0.001
Parity **Primi** **Multi**	 39(48.8%) 41(51.2%)	 33(41.3%) 47(58.8%)	 0.9	 0.34
Family history **+ve** **-ve**	 72(90%) 8(10%)	 0 80(100%)	 130.9	 <0.001

Table (2): Comparison of Lep rs7799039 genotypes between the studied Groups

	group I (GDM) N=80	group 2 N=80	X2	P value	OR(CI)
Rs7799039					
GG*	15(18.8%)	46(57.5%)			
AG	27(33.8%)	20(25%)	27.9		4.1(1.8-9.4)
AA	38(47.5%)	14(17.5%)		<0.001	8.3(3.6-19.4)
Alleles					
G*	57(35.6%)	112(70%)			
A	103(64.4%)	48(30%)	37.9	<0.001	4.2(2.6-6.7)

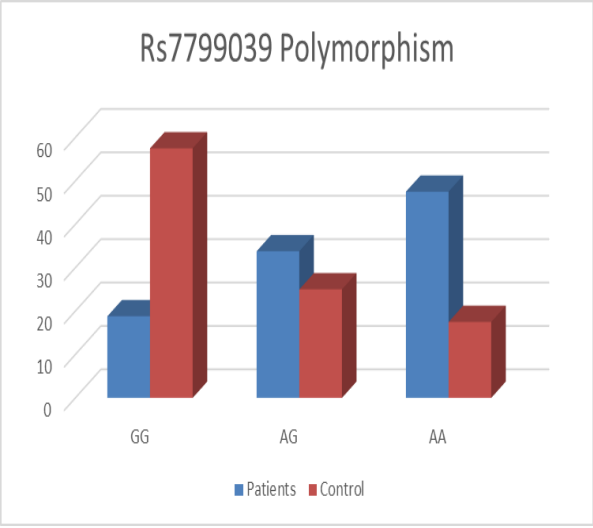


Fig. (1): Genotype distribution of theLeprs7799039 (G/A) polymorphism between two studied groups

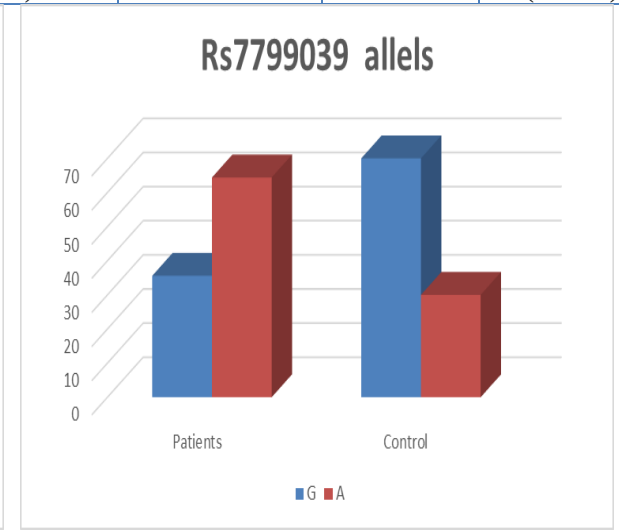


Fig. (2): Allelic distribution of theLeprs7799039 (G/A) polymorphism between two studied groups

correlation (r=-0.9 and P<0.001) between plasma leptin levels and fasting insulin in group I as shown in figure 3.

Discussion

Gestational diabetes mellitus (GDM), one of the most common pregnancy complications, is defined as the first recognition of glucose intolerance during pregnancy. Adverse pregnancy outcomes of GDM impact on both mothers and their offspring during and after pregnancy (31). Insulin resistance is an important characteristic of both Type II Diabetes and GDM. Several adipokines including leptin, visfatin, adiponectin and resistin are reported to be involved in development of insulin resistance (32).The aim of this work was to evaluate LEP-2548 G/A single nucleotide polymorphism [SNP] at position -2548 G/A and plasma leptin level as risk factors for GDM.

In our current study, There was a statistically significant difference between the two studied groups regarding age,serum FBG,HBA1C,SBP, DBP,2HPP, TC,TG ,LDLc, HDLc fasting insulin, HOMA-IR, baby WT, plasma leptin ,family history of DM and pre pregnancy BMI. This result was in agree with Mei Yang et al.,2016 (33) who reported that the pregnant women with GDM had a higher age, pre-pregnant body mass index (BMI), systolic blood pressure, diastolic blood pressure and fasting blood glucose (P <0.001). Furthermore, the women with impaired fasting glucose (IFG) had significantly higher fasting plasma leptin levels than normal glucose tolerance (NGT) group.(34)also showed that GDM is associated with insulin resistance and adverse maternal health outcomes such as gestational hypertension and pre-eclampsia, and neonatal outcomes including hyperinsulinaemia, macrosomia, shoulder dystocia, later life risk of obesity and T2DM. Khalid S. et al.,(35) reported that the diabetic patients had higher BMI, serum triglyceride and HbA1c values when compared to non-diabetic subjects. Whereas, had lower HDL level. This study revealed that obesity and dyslipidemia were high among diabetic patients.

In our study plasma leptin level was significantly higher in GDM than control group .concerning this It has been reported that the levels of leptin were increased (36, 37, 38), decreased (39) or unchanged(40) in GDM. Noureldeen, *et al.*,(41) reported that plasma leptin levels did not significantly change at 2nd trimester but decrease at 3rd trimester among GDM women(42).In our current study there was significant difference regarding the Leprs7799039 genotype distribution between the two studied groups, with increased frequency of the AA and AG genotypes and A allele in the patient(GDM) group and increased GG genotype and G allele frequency in the control group.

The result also showed that the AA genotype of Leprs7799039(G/A) increases the risk of GDM by 8.3- fold and AG genotype increases the risk by 4.1-fold, while the A allele increases the risk by 4.2-fold,with highest plasma leptin in AA genotype this result was in agree with Julie Anna et al.,(42) who reported that the significantly higher risk for gestational diabetes mellitus was observed in the presence of an allele (AA and AG genotypes) against carriers of GG genotype (OR=2.84, 95%CI 1.14-7.07, p=0.02). There is a significant risk of diabetes mellitus associated to A allele (OR=1.79, 95%CI 1.02-3.14, p=0.03).

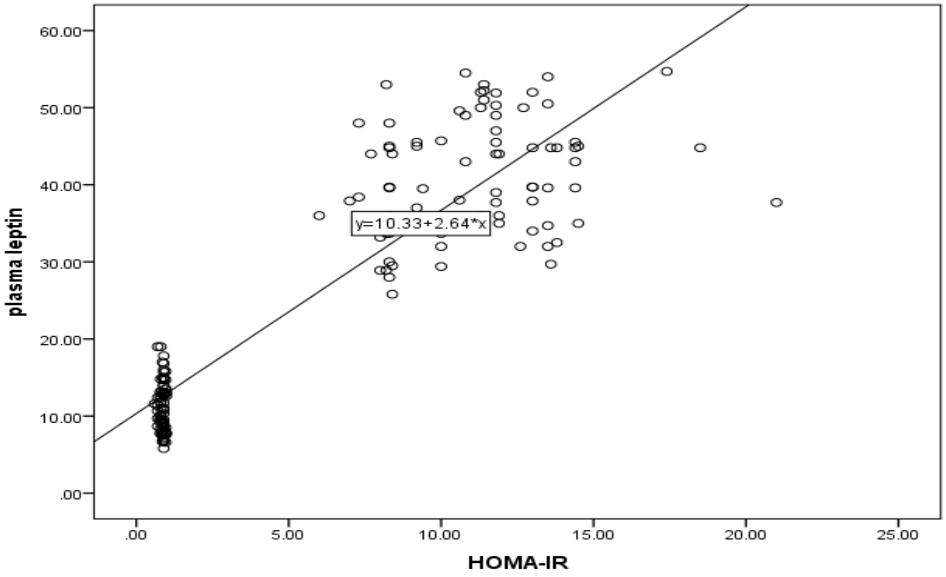
Table (3): Biochemical parameters of the studied patients with gestational diabetes in different genotypes of Leprs7799039 (G/A).

	GG	AG	AA	**F test**	**P value**	
Age (years)	25.1±4.6	25.9±4.6	27.7±5.7	4.8	0.009	I&III:0.003 II&III:.047
Gestational age (weeks)	35.2±4.1	35.7±3.3	36.4±2.6	1.9	0.154	--------
BMI (Kg/m^2)	22.8±3.3	24.9±4	25.5±3.6	8.6	<0.001	I&II:0.003 I&III:<0.001
SBP(mmHg)	120.4±16.5	131.1±19	128.1±17.5	5.4	0.005	I&II:0.002 I&III:0.02
DBP(mmHg)	81.6±10.2	86.6±11.4	86.2±10.6	3.9	0.02	I&II:0.016 I&III:0.02
FBS(mg/dl)	117.8±54	163.7±68.7	182.6±64.4	16.4	<0.001	I&II:<0.001 I&III:<0.001
2HPP(mg/dl)	126.2±67.8	178.2±81.1	203.7±77.7	15.4	<0.001	I&II:<0.001 I&III:<0.001
HBA1$_C$%	5.8±1.3	6.6±1.5	7.1±1.6	11.2	<0.001	I&II:0.005 I&III:<0.001
HDLc(mg/dl)	44.5±7.1	38.9±8.6	36.2±7.4	17.5	<0.001	I&II:<0.001 I&III:<0.001
Total Cholesterol(mg/dl)	178.5±17.6	197.5±33.6	201.4±31.6	11.1	<0.001	I&II:0.001 I&III:<0.001
LDL-C (mg/dl)	111.8±17.7	131.8±34.6	136±32.1	11.9	<0.001	I&II:<0.001 I&III:<0.001
F.Insulin (uIU/ml)	7.9±7.1	13.4±8.6	16.7±7.9	17.9	<0.001	I&II:<0.001 I&III:<0.001 II&III:0.04
HOMA-IR	3.1±4.2	6.7±5.6	8.7±5.2	18.2	<0.001	I&II:<0.001 I&III:<0.001
Baby WT (Kg)	3.3±0.4	3.7±0.7	3.9±0.6	14.3	<0.001	I&II:0.001 I&III:<0.001
Plasma leptin(ng/ml)	16.8±9.3	25.4±14.1	38±16.9	33.9	<0.001	I&II:0.001 I&III:<0.001 II&III<0.001

*K test

Table(4): Multivariate logistic regression for risk of GDM

	B	**P value**	**OR**	**CI**
Genotype **GG*** **AG** **AA**	 0.6 1.6	 0.465 0.013	 2.1 9.06	 0.119-2.6 2.06-10.7
Plasma leptin(ng/ml)	3.6	<0.001	32.1	6.1-169.5
Birth weight (Kg)	3.1	<0.001	22.7	6.5-79
HOMA-IR	2.2	<0.001	9.2	3.01-28.3



		Plasma leptin
	r	P value
HOMA IR	0.9	<0.001
F insulin	0.93	<0.001

r = 0.9 p value <0.001

Fig. (3): Correlation coefficient between serum Plasma leptin levels & HOMA-IR in group I.

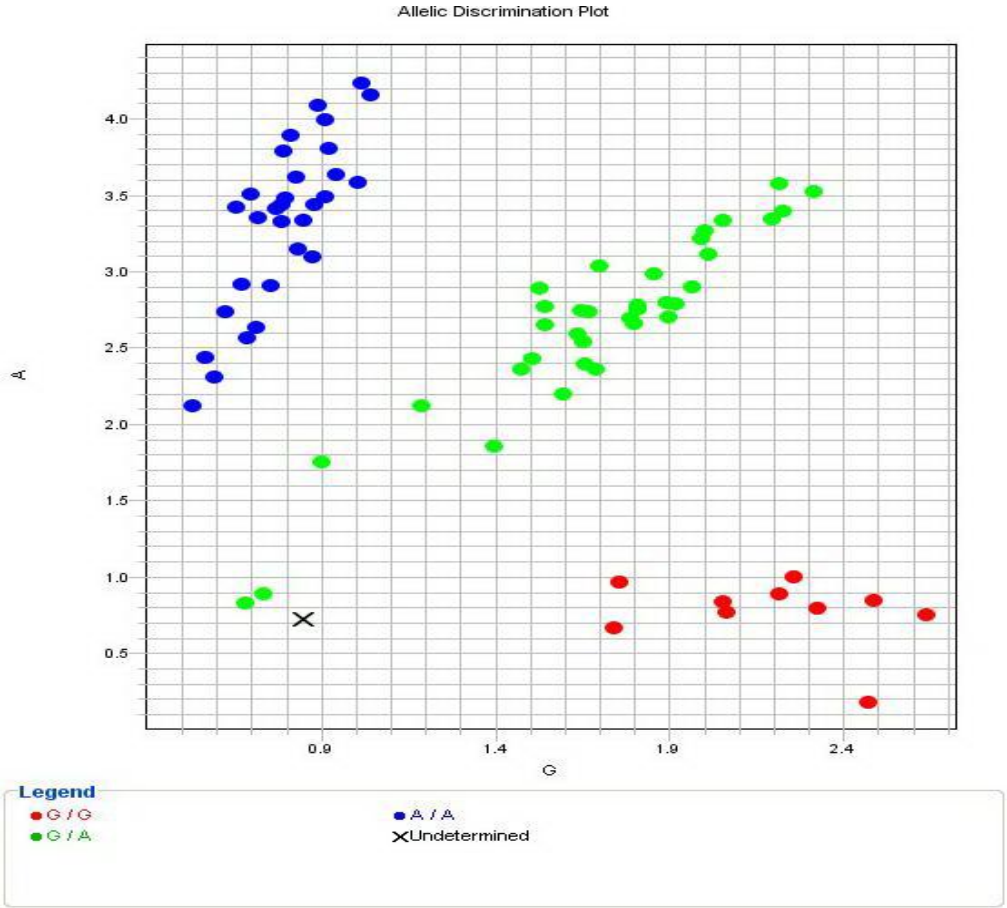


Fig. (4): Allelic discrimination plot showing AA, AG and GG genotypes of Leprs7799039 (G/A).

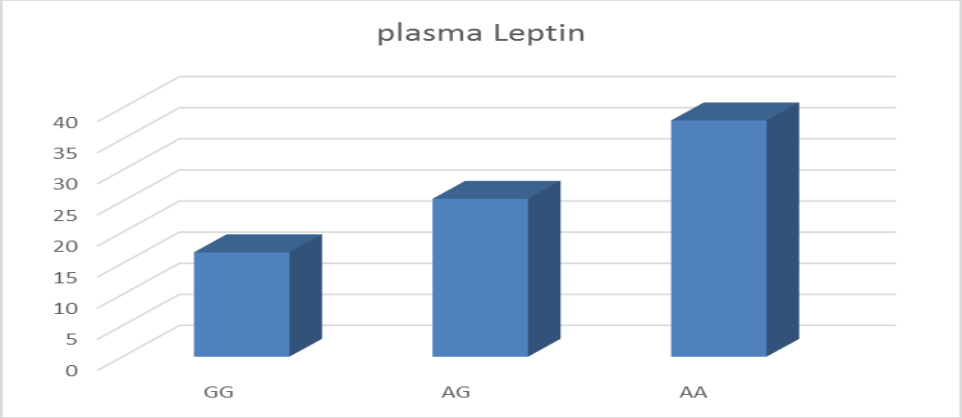


Figure (5): Association of plasma leptin level with LEP 2548 G/A polymorphism.

Hoffsted et al. (18) showed that individuals with the AA genotype of rs7799039 had higher serum leptin concentrations than the AG or GG genotypes carriers. A Czech study with 48 GDM and 53 controls showed that AA and AG genotype carries of LEP-2548 G/A polymorphism had a significantly higher risk for GDM against those carrying GG genotype (33).In -2548 G/A polymorphism, an adenine replaces guanine in nucleotides -2548 upstream from the ATG start site at 5′ region of leptin gene promoter. This polymorphism affects the expression of leptin and its secretion from adipose tissue (43).The placenta produces leptin. Leptin levels rise during pregnancy and fall after childbirth (44).A meta-analysis including 18 observational studies found that leptin concentrations were significantly higher in GDM patients compared to controls (34).

The leptin (LEP) G2548A polymorphism has been associated with increased leptin production and plasma secretion from adipocytes (18). It was proved that leptin gene expression and production are markedly elevated in placenta of diabetic women treated with insulin, hyperinsulinoma and hypoxia. Insulin and hypoxia act as stimuli of the human leptin transcription by two different regulatory mechanisms. It has been published that hypoxia induces leptin transcription by a hypoxia-inducible-factor-1 (HIF-1) dependent and insulin response element mechanisms (42).

In our study; Multivariate logistic regression for risk of GDM showed that the most common risk factor is plasma leptin OR; 32.1 (6.1-169.5), followed by Birth weight OR; 22.7 (6.5-79), HOMA-IR OR; 9.2 (3.01-28.3), AA genotype OR; 9.06 (2.06-10.7).A larger prospective cohort study found that hyperleptinaemia was predictive of increased risk of GDM. There was a strong linear correlation, with each 10 ng/mL increase in leptin concentration associated with a 20% increase in GDM risk (45).The adipokines; adiponectin, leptin, TNF-α and adipocyte fatty acid-binding protein (AFABP) are increased in obesity and pregnancy and are prime candidates for direct involvement in the pathophysiology of GDM (46).

Leptin secreted from the placenta may contribute to regulation of fetal growth independent of maternal glucose levels. Leptin may contribute to GDM pathophysiology by suppressing insulin secretion from pancreatic beta cells (34).

The present study showed that There was a significant positive correlation (r=-0.9 and P<0.001) between plasma leptin levels and insulin resistance represented by HOMA-IR in group I. Also, there was aa significant positive correlation (r=-0.9 and P<0.001) between plasma leptin levels and fasting insulin in group I. This result was in agree with Mei Yang et al., (33) who reported that the fasting plasma leptin levels were positive correlated with fasting plasma insulin, HOMA-IR.

Conclusion: Our results concluded that AA genotype and A allele of Lep2548 (G/A) polymorphism are risk factors for Gestational Diabetes Mellitus (GDM).Also, plasma leptin level might be used for screening of the early detection of GDM.

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