A study on serum Leptin levels and models of Insulin resistance in Gestational Diabetes Mellitus (GDM)

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Abstract

Gestational diabetes mellitus (GDM) is characterized by diminished glucose tolerance that manifests during pregnancy. Leptin, a vital adipokine governing several physiological processes, including insulin sensitivity, plays a pivotal role. Insulin resistance stands as the principal factor underlying the development of GDM. This study aims to assess and compare serum leptin levels and various insulin resistance (IR) models between GDM cases and normoglycemic pregnant women. Furthermore, we seek to determine whether leptin and IR models hold predictive value for GDM.A cross-sectional study was conducted, enrolling one hundred GDM cases and one hundred normoglycemic pregnant women. Fasting blood samples were collected for the analysis of serum leptin, insulin, and C-peptide using ELISA. Appropriate statistical analyses were performed employing GraphPad InStat 3.Fasting C-peptide levels were significantly higher in GDM cases ($p=0.0014$). Conversely, fasting serum insulin and leptin levels exhibited no significant differences in GDM patients ($p=0.6968$ and $p=0.213$, respectively). Comparative analysis of IR models between cases and controls revealed significantly lower HOMA B cell and HOMA 1% B cell (insulin-based) values, along with significantly higher HOMA B cell and HOMA 1% B cell (C-peptide-based) values in cases ($p<0.0001$).In summary, our findings suggest that leptin levels were not significantly altered in GDM, while C-peptide and C-peptide-based insulin resistance models were elevated. Both leptin and various insulin resistance models did not emerge as reliable markers for predicting GDM.

Keywords: leptin; insulin; C-peptide; Insulin resistance; GDM

Introduction

Gestational diabetes mellitus (GDM) represents a pregnancy-related complication characterized by impaired carbohydrate tolerance, which either manifests or is first recognized during pregnancy (Metzger et al.,2007). It arises due to reduced insulin sensitivity, leading to altered metabolic effects such as increased postprandial free fatty acids (FFAs), heightened hepatic glucose production, and elevated blood glucose levels. Adipose tissue functions as an endocrine organ, secreting various adipokines that facilitate communication between adipose tissue and other organs. Among these adipokines, leptin plays a crucial role, mediating a wide range of functions, including the regulation of lipid and carbohydrate metabolism, insulin sensitivity, atherosclerosis, angiogenesis, and more. Leptin levels have been reported to be altered in GDM, with some studies indicating increases or decreases (McLachlan., 2006) (Festa et al., 1999). Nevertheless, these reports are inconsistent, and a definitive conclusion has yet to be reached. Insulin resistance in GDM has been linked to elevated leptin levels (Laivuori et al., 2000).

Insulin resistance stands as the central factor contributing to GDM development. Reduced maternal insulin sensitivity before pregnancy, coupled with an inadequate insulin response, underlie the onset of GDM. Clinical study reports suggest that elevated leptin levels result from the upregulation of the leptin gene due to insulin resistance and hyperinsulinemia (Laivuori et al., 2000). It has also been observed that leptin influences whole-body insulin sensitivity by regulating insulin-mediated glucose metabolism in skeletal muscle and controlling hepatic gluconeogenesis (Cohen B, 1996), (Rossetti et al., 1997). Furthermore, leptin has been found to exert an inhibitory effect on insulin secretion (Ceddia et al., 2002). Consequently, it is justifiable to investigate the association between Leptin gene polymorphism, circulating Leptin levels, and insulin resistance in GDM.

The objectives of this study were to compare leptin levels and various biochemical parameters in GDM patients with those in normal pregnant women.

Material and method

This study was conducted at the Central Research Laboratory of K.S. Hegde Medical Academy and the Department of Obstetrics and Gynecology at K.S. Hegde Charitable Hospital of Nitte University in Mangaluru, Karnataka, India.

One hundred GDM patients diagnosed on the basis of 75 gm oral glucose tolerance test (OGTT) according to the ADA 2016 criteria were included as cases. Additionally, one hundred pregnant women with matching gestational age and BMI, and normal glucose tolerance, were selected as the control group. Exclusion criteria encompassed multiple pregnancies, known pre-gestational diabetes, pregnancies complicated by major fetal malformations, or known major cardiac, renal, or hepatic disorders, as well as pregnancy-induced hypertension (PIH).

The study obtained institutional ethics committee approval, and written informed consent was obtained from all participating patients. Eligible patients were recruited, and venous blood samples, amounting to five milliliters, were collected for biochemical analysis. Fasting leptin, insulin, and C-peptide levels were assessed using ELISA. Insulin resistance was calculated utilizing the homeostasis model assessment (HOMA) model, and both insulin and C-peptide-based insulin resistance models were constructed using the following formulas:

Statistical analysis

The statistical analysis was performed using SPSS version 23.0. Categorical data was presented as percentages, while continuous data was expressed as mean ± standard deviation (SD). To compare biochemical parameters between the cases and controls, the Mann-Whitney U test was utilized. Spearman's correlation test was employed to explore the relationships between biochemical parameters. A 'p' value of less than 0.05 was considered statistically significant. Receiver Operating Characteristic (ROC) curves were constructed to evaluate the potential of leptin levels and insulin resistance (IR) models as indicators for predicting GDM.

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

Results

The study included GDM patients with a mean age of 29.62 ± 4.3 years and normal pregnant women with an average age of 27.08 ± 3.73 years. The mean BMI for both groups was 25.78 ± 6.84 kg/m2 and 25.86 ± 5.86 kg/m2, respectively. The gestational age of the subjects was 25.87 ± 1.21 weeks for GDM cases and 26.1 ± 1.54 weeks for normal pregnancies.

Comparison of their biochemical parameters revealed a significantly higher fasting blood sugar (FBS) level in GDM cases ($p < 0.0001$). Additionally, fasting C-peptide levels were significantly elevated in GDM cases ($p = 0.0014$). However, fasting serum insulin and leptin levels exhibited no significant differences in GDM patients ($p = 0.6968$ and $p = 0.213$, respectively).

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(fig1).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 between insulin resistant cases (HOMA IR>2.4) compared to GDM patients with normal insulin sensitivity. Serum C peptide was significantly higher in IR cases (table 3). Correlation studies showed a significant negative correlation between FBS and leptin ($r=-0.232$ $p=0.0237$).

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 4).

ROC were 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 52.7 ng/ml (figure 2). 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 3). However other IR models were found to be poor predictors of GDM.

Table 2: Depicting metabolic parameters in cases and controls

Parameter	GDM (n=100)	Control (n=100)	P value
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
C-peptide(nmol/L)	2.17±1.71	1.57±1.55	0.0014*
Leptin(ng/ml)	57.33±23.96	63.11±25.46	0.213

*p value significant

Table 3: Comparison of Biochemical parameters in GDM cases with and without IR

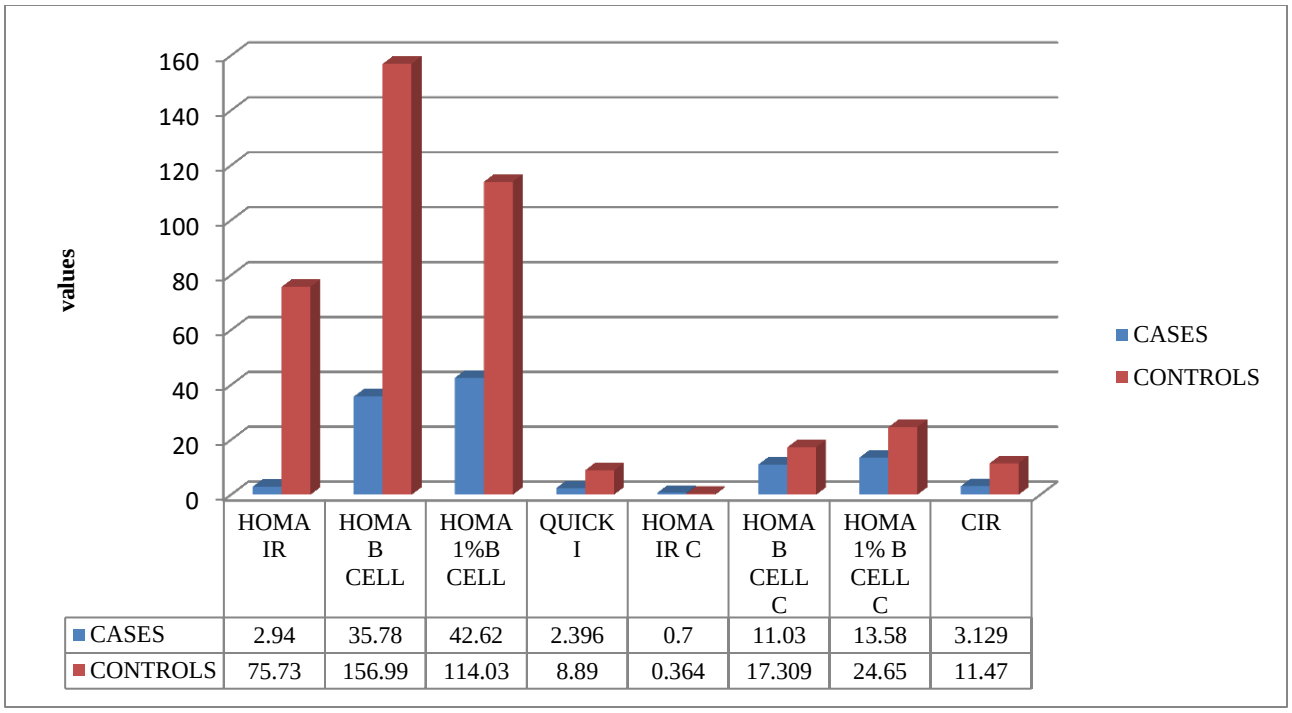
Parameter	Insulin resistant cases	Cases with Normal Insulin sensitivity	p value
C-peptide(nmol/L)	3.15±1.85	1.72±1.44	0.0001★
Leptin(ng/ml)	56.31±24.45	57.79±23.91	0.99

*p value significant

Table 4: 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



	HOMA IR	HOMA B CELL	HOMA 1%B CELL	QUICK I	HOMA IR C	HOMA B CELL C	HOMA 1% B CELL C	CIR
CASES	2.94	35.78	42.62	2.396	0.7	11.03	13.58	3.129
CONTROLS	75.73	156.99	114.03	8.89	0.364	17.309	24.65	11.47

Fig 1: Comparison of IR Models in GDM patients & Normal Pregnant Women

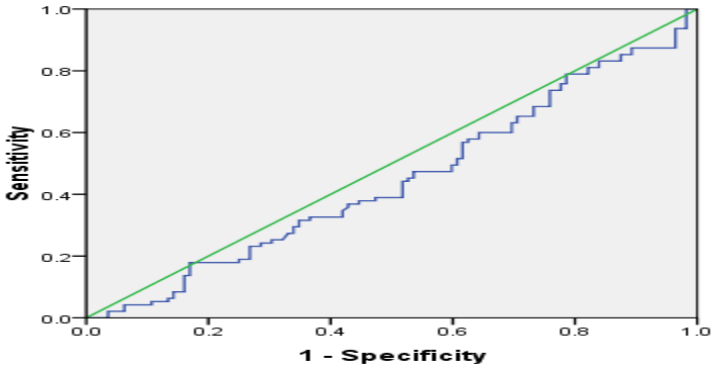


Fig. 2: ROC for leptin as a marker for GDM

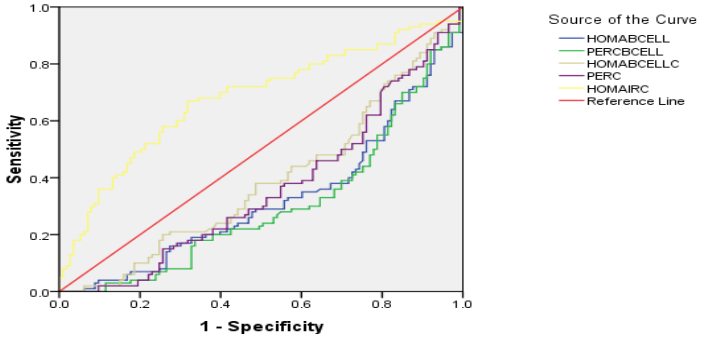


Fig. 3: ROC of IR models for prediction of GDM

Discussion

Leptin levels were found to be statistically insignificant and C-peptide levels were significantly elevated in GDM cases, as indicated in Table 2. During pregnancy, maternal leptin concentrations typically increase two to threefold compared to non-pregnant levels, with the peak occurring around the 28th week of gestation (Schubring et al., 1998). Given leptin's potential role in maternal metabolism and glucose regulation, it has been suggested as a valuable marker for predicting GDM. However, existing reports on maternal leptin levels in GDM have yielded conflicting results, with some studies reporting elevated leptin levels (Kautzky-Willer et al.,2001) (Gao XL,2008) reduced levels, or no significant differences compared to controls (Simmons & Breier, 2002) (Mokhtari et al.,2011) (Fruscalzo et al., 2015). Notably, Noureldeen et al. found no significant variation in leptin levels during the second trimester but observed reduced levels during the third trimester among GDM patients (Noureldeen et al., 2014) In a cohort study, Qiu et al. demonstrated that a 10 ng/ml increase in leptin levels during early pregnancy was associated with a 20% increase in GDM risk(Qiu et al., 2004).

The disparities in these studies may be attributed to several factors. Pregnancy is known to induce leptin resistance, linked to impaired leptin signaling. One potential function of increased maternal leptin levels is to facilitate the mobilization of maternal fat stores, enhancing their availability and supporting the trans-placental transfer of lipid substrates (Hauguel-de et al.,2006). Mounting evidence suggests that the placenta, rather than adipose tissue, is the primary contributor to plasma leptin levels (Bi et al.,1997), and human placental gene regulation may differ from that of adipose tissue. Furthermore, the fetus may contribute to maternal leptin levels as early as the second trimester (Christou et al.,2002). While most studies report elevated leptin concentrations in GDM (Kautzky-Willer et al., 2001), hyperleptinemia in early pregnancy appears to predict an increased risk of developing 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 elevated ($p = 0.0014$) among cases (Table 2). Comparing insulin resistance (IR) models between cases and controls, insulin-based models (HOMA B cell and HOMA 1% B cell) were significantly lower, while C-peptide-based models (HOMA B cell, HOMA 1% B cell) were significantly higher in our study ($p < 0.0001$). Notably, C-peptide-based IR models (HOMA IR-C and CIR) were significantly higher ($p < 0.0001$) in cases compared to controls (Figure 1). Given that C-peptide is a well-established marker of endogenous insulin secretion and C-peptide-based IR models are robust indicators of insulin resistance, we conclude that GDM patients exhibit higher IR compared to normal glucose-tolerant pregnant women. However, ROC analysis indicated that none of the IR models, as well as leptin, were strong predictive markers for GDM, except for HOMA IRC, which had an AUC of 0.67 (Fig 3 & Fig 2).

Furthermore, compared to the normal glucose-tolerant (NGT) group, higher leptin levels were observed in the impaired fasting glucose (IFG) group, consistent with previous research (Honnorat et al., 2015) (Endo et al., 2006). The IFG and impaired glucose tolerance (IGT) groups exhibited significantly higher fasting insulin levels, HOMA-IR, and lower QUICKI compared to the NGT group. These findings aligned with positive and negative correlations between plasma leptin levels and HOMA-IR and QUICKI, respectively. While these correlations are supported by some studies (Ozcimen et al., 2008), (Yilmaz et al., 2010], they contradict others (Al-Daghri et al., 2002). Notably, positive correlations between plasma leptin concentrations and maternal pre-pregnant BMI were consistent with previous findings in both GDM and NGT groups.

The exponential increase in the incidence of diabetes has termed it as metabolic epidemic in the developing world (Usah et al 2023). Some research suggests that leptin plays a significant role in regulating whole-body glucose homeostasis (Havel et al., 1996) (Schubring et al., 1998), with positive correlations observed between measures of adiposity and plasma leptin concentrations (Laivuori et al.,2000). Pregnancy-related changes in maternal fat stores and glucose metabolism contribute to increased maternal leptin levels (Donahue et al., 1999), with this increase attributed to upregulated adipocyte leptin synthesis in the presence of growing insulin resistance and hyperinsulinemia during the latter half of pregnancy. Studies have shown that leptin directly influences whole-body insulin sensitivity by modulating insulin-mediated glucose metabolism in skeletal muscle and regulating hepatic gluconeogenesis (Rossetti et al., 1997). Additionally, leptin has been found to acutely inhibit insulin secretion. Large-scale epidemiological studies have demonstrated positive associations between plasma leptin concentrations and insulin resistance in both men and non-pregnant women.

Conclusion

In summary, the study findings suggest that leptin levels did not exhibit a significant difference, while C-peptide levels and C-peptide-based insulin resistance models were notably higher in GDM patients. Notably, there was a significant negative association between leptin and insulin levels, as well as HOMA-IR, and a positive

correlation with QUICKI in cases with insulin resistance. Nevertheless, both leptin and various insulin resistance models did not prove to be effective markers for detecting GDM.

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Conflicts of interest: None

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