SERUM LEPTIN LEVELS AND INSULIN RESISTANCE MODELS OF GESTATIONAL DIABETES MELLITUS

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Abstract

Introduction: Gestational diabetes mellitus (GDM) is defined by reduced glucose tolerance that appears or is first noticed during pregnancy. Leptin is a crucial adipokine that regulates a variety of processes, including insulin sensitivity, The main contributing factor to the emergence of GDM is insulin resistance. Objective of the study is to compare leptin levels and various insulin resistance (IR) models between GDM cases and normoglycemic pregnant women. Aim is to also find out whether leptin and IR models can be predictors of GDM.

Methodology: The cross-sectional study was carried out with the inclusion of hundred each GDM and normoglycemic pregnant women. Fasting blood samples were drawn for analyzing serum leptin, insulin, C-peptide by ELISA. Suitable statistical tests were applied using Graphpad instat 3.

Results: Fasting C peptide was significantly higher in cases ($p=0.0014$). Fasting serum insulin and leptin levels were insignificantly low in GDM patients ($p=0.6968$ and $p=0.213$). 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.

Conclusion: It could be concluded from the study that, leptin was insignificantly low,C-peptide and C –peptide based insulin resistance models were elevated in GDM patients. Leptin as well as various insulin resistance models were not good markers to predict GDM.

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

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DOI: 10.48047/ecb/2023.12.si10.00204

Background:
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, report available are conflicting and fact is yet to be established. Insulin resistance in GDM has been associated with elevated leptin levels [4].

Insulin resistance is the key factor in the development of GDM. Reduced maternal pregravid insulin sensitivity coupled with inadequate insulin response are the causative factors for GDM.

Clinical study reports suggest that elevation of leptin levels are due to upregulation of leptin gene due to insulin resistance and hyperinsulinemia [4]. It has been reported that leptin affects whole body insulin sensitivity by regulating insulin mediated glucose metabolism by skeletal muscle as well as hepatic regulation of gluconeogenesis [5,6]. Leptin has been found to have inhibitory effect on insulin secretion [7]. Hence it is justifiable to study the association of Leptin gene polymorphism, circulating Leptin levels and insulin resistance in GDM.

Objectives of the Study was to compare leptin levels as well as other biochemical parameters in GDM patients as compared to normal pregnant women

Methodology:
The **study was conducted in** Central Research Laboratory of K.S.Hegde Medical academy and Department of OBG, K.S.Hegde Charitable Hospital of Nitte University, Mangaluru, Karnataka, India.

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 pregnancy, known pre-gestational diabetes, pregnancies complicated by major fetal malformations or known major cardiac, renal or hepatic disorders, PIH

Institutional Ethics committee approval was obtained and Written Informed Consent was taken from patients.
Patient who are fulfilling the study criteria were recruited. Five milliliters of venous blood samples were drawn from the recruited patients for biochemical analysis. Fasting leptin, insulin and C-peptide were assayed by ELISA.

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

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). 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. A 'p' value <0.05 was regarded as statistically significant. 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/m^2 and 25.86±5.86 kg/m^2 respectively. Gestational age of the subjects were 25.87± 1.21 wks and 26.1±1.54 wks respectively.

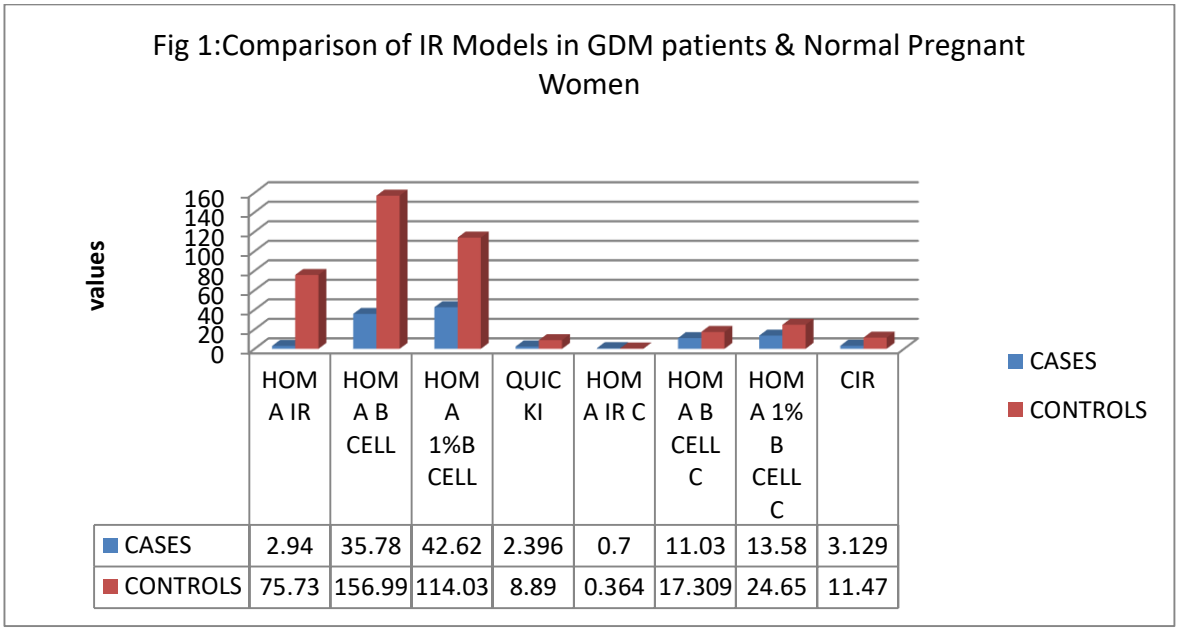
Comparison of their biochemical parameters showed significantly high (p<0.0001) FBS in GDM cases. Fasting C peptide also was significantly higher in cases (p=0.0014). Fasting serum insulin and leptin levels were insignificantly low in GDM patients (p=0.6968 and p=0.213).

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).

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



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).

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

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).

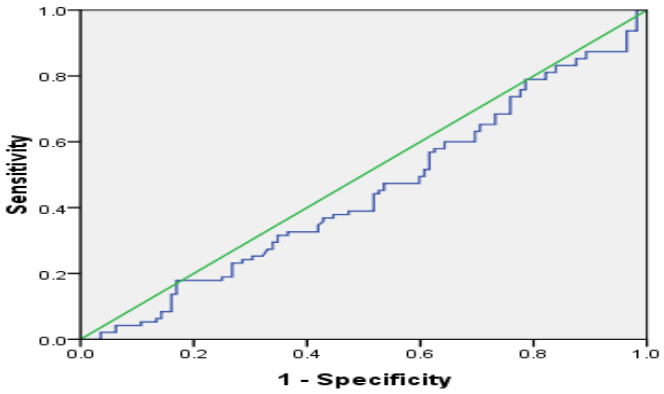
Table 4: Correlation of Leptin levels with IR Models

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

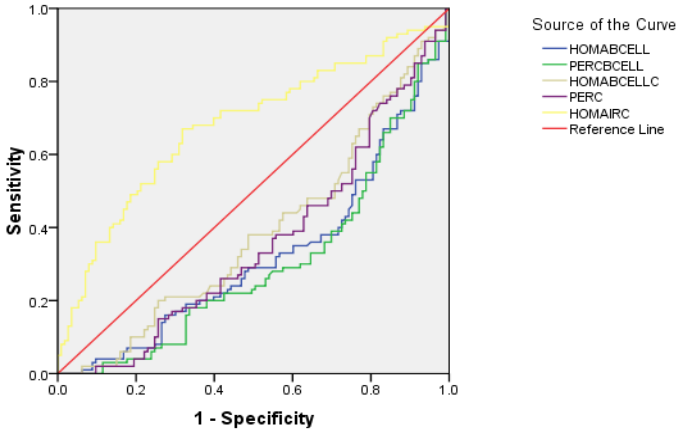
ROC were constructed to assess the utility of leptin as a marker of GDM. Area under the curve was 0.446, with a sensitivity of 49.5% and specificity of 60.7% at a cut off value of leptin being 52.7 ng/ml(fig 2).

Fig 2: 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 3). However other IR models were found to be poor predictors of GDM.

Fig 3: ROC of IR models for prediction of GDM



Discussion

Leptin was insignificantly low and C-peptide was significantly higher in GDM cases(table 2).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 [8]. 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 [9-11], diminished leptin levels [3] or insignificant differences in leptin levels among GDM patients compared to controls [12-14]. 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 [15]. 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 [16].

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 [9]. 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 [17]. There is strong evidence that suggest placenta is the main contributor of plasma leptin rather than the adipose tissue [18]. 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 [19]. Most studies have found increased leptin concentrations in GDM [9]. Moreover, hyper leptinaemia 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 (table 2).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(fig 1).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. However, ROC analysis showed that none of the IR models as well as leptin were good predictive markers of GDM except for HOMA IRC, with AUC 0.67(fig 3) and (fig 2).

Compared with the NGT group, higher leptin levels were found in the IFG group, consistent with the previous study [20,21]. 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. A positive and negative correlations were found between plasma leptin levels and HOMA-IR and QUICKI respectively. These correlations were confirmed in some studies [22,23], but contradicted in a few studies [24]. The observed positive correlations between plasma leptin concentrations and the maternal pre-pregnant BMI were in accordance with many previous studies both in GDM group and NGT group.

Some studies suggest that leptin also has a main effect on the regulation of whole-body glucose Homeostasis [7,26]. Some studies demonstrated a positive correlation between direct and indirect measures of adiposity with plasma leptin concentrations [27]. In pregnant women with changes in maternal fat stores and glucose metabolism, leptin increases [28]. Maternal leptin concentration increases 2-3 times above the nonpregnant concentration with the peak around 28 weeks of gestation. Studies suggest that increasing maternal plasma leptin may result from an upregulation of adipocyte leptin synthesis in the presence of increasing insulin resistance and hyperinsulinemia in the second half of pregnancy. 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 [5,6]. 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 nonpregnant women.

Conclusion

It could be concluded from the study that, leptin was insignificantly low, C-peptide and C –peptide based insulin resistance models were elevated in GDM patients. Leptin showed a significant

negative correlation with insulin levels, HOMA-IR and positive correlation with QUICKI in IR cases. Leptin as well as various insulin resistance models were not good markers to detect GDM.

Acknowledgements: Sincere thanks to Dr Suchetha Kumari, Molecular Genetics Laboratory Incharge for the permission to carry out the work.

Funding: Research Society for The Study of Diabetes in India

Conflicts of interest: None

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