Original Article

Insulin Sensitivity, Islet Cell Function, and Incretin Axis in Pregnant Women With and Without Gestational Diabetes Mellitus

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Abstract

Introduction: The aim of this study was to compare insulin sensitivity, islet cell function, and incretin axes in pregnant subjects with GDM and normal healthy controls. **Methods:** Pregnant women at 24 to 28 weeks of gestation were subjected to a 75 g oral glucose tolerance test (OGTT). Samples for glucose, insulin, glucagon, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) were collected at 0, 30, 60, and 120 min during the OGTT. The Matsuda index (MI) and insulin secretion and sensitivity index-2 (ISSI-2) were assessed. The glucagon suppression index (GSI) was calculated along with the area under the curve (AUC) for glucose, insulin, glucagon, GLP-1, and GIP. **Results:** A total of 48 pregnant women (25 GDM and 23 controls) were finally analysed. The MI and ISSI-2 were low in the GDM group [4.31 vs. 5.42; $P = 0.04$], [1.99 vs. 3.18, $P \leq 0.01$] respectively). Total $AUC_{glucagon}$ was higher in the GDM group (7411.7 vs. 6320.1, $P = 0.02$). GSI_{30} was significantly lower in the GDM group (-62.6 vs. -24.7, $P = 0.03$). Fasting GLP-1 levels were low in GDM women (17.3 vs. 22.2, $P = 0.04$). The total AUC_{GLP-1} positively correlated with total GSI in the GDM group. **Conclusion:** Asian-Indian GDM women have high insulin insensitivity, islet cell dysfunction, and low fasting GLP-1. Incretin axis dysfunction plays a potential role in their islet cell dysfunction.

Keywords: Alpha cell, beta cell, diabetes in pregnancy, glucagon, glucose-dependent insulinotropic polypeptide, glucagon-like peptide-1, insulin secretion and sensitivity index-2

Introduction

Gestational diabetes mellitus (GDM) is a common metabolic disorder that develops during pregnancy and is classically defined as "carbohydrate intolerance first identified during pregnancy."[1] Globally, the prevalence of GDM varies from 1% to 30%, with significant regional variation.[2] GDM predisposes women to type 2 diabetes mellitus (T2DM), and the two conditions share a similar spectrum of metabolic alterations.[2]

Pregnancy is a diabetogenic state that is linked to an increase in insulin resistance that begins in the second trimester and peaks in the third trimester, eventually reaching T2DM levels.[3] Insulin insensitivity observed during pregnancy is caused by a combination of placental hormones' insulin-desensitizing effects and increased maternal adiposity.[4] Pancreatic beta-cells must significantly increase insulin secretion and compensate for the physiologic insulin resistance in pregnant women to maintain normal glucose homeostasis. GDM arises in women when this beta-cell compensatory response is inadequate.[5] Asian-Indians have a distinct metabolic profile than Caucasians, and they are ethnically prone to developing T2DM at an earlier age and have a lower body mass index (BMI).[6] Also, Asians have lower pancreatic beta-cell mass and higher insulin resistance.[7,8]

Glucagon secreted from the alpha cells regulates glucose metabolism in conjunction with its biological antagonist

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insulin.[9] Incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) regulate the entero-insular axis, involving the intestine and endocrine pancreas.[10] Both glucagon dysregulation and incretin dysfunction play a vital role in the etiopathogenesis of T2DM.[11] GDM, being a prediabetic state with a similar spectrum of metabolic alterations, will probably have similar changes. Very few studies have evaluated these axes in GDM women, and the majority of them were performed on Caucasians.[12–19] Similar research in the Asian-Indian population is limited.

Though a few studies have identified insulin insensitivity and beta-cell dysfunction in Asian-Indian GDM women,[20–23] glucagon and the incretin axes have not been studied in this population. Therefore, we designed a cross-sectional study to assess insulin sensitivity, islet cell function, and incretin axis in Asian-Indian women with GDM versus normoglycemic healthy pregnant women.

Materials and Methods

Study setting and subjects

This prospective, observational study was carried out in the Department of Endocrinology of a tertiary care centre in South India from July 2018 to July 2020 after obtaining approval from the Institute Ethics Committee (JIP/IEC/2018/0133) and was conducted according to the principles of the Helsinki Declaration II.

Singleton pregnant women aged 18–35 years in the second trimester of pregnancy (24–28 weeks of gestation) were recruited to the study from the antenatal outpatient clinic of the Obstetrics and Gynecology department of the institute. Consecutive pregnant women fulfilling the inclusion criteria were screened with an oral glucose tolerance test (OGTT) using 75 g of anhydrous glucose (82.5 g glucose monohydrate) until 25 GDM women were included in the study group. GDM was diagnosed using the International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria.[24] Women were excluded if they had been diagnosed with overt diabetes in pregnancy (fasting plasma glucose ≥126 mg/dL and/or 2-h plasma glucose in a 75 g OGTT ≥200 mg/dL and/or glycated haemoglobin [HbA1c] ≥ 6.5%) or were complicated with other diseases such as connective tissue disorder, renal disease, liver disease, cardiovascular disease, and respiratory disease, or had a history of pancreatectomy or any other gastrointestinal surgery. None of the women had previously been treated with antidiabetic agents, including insulin. Twenty-five women with normal glucose tolerance (NGT) having all plasma glucose values below the cut-off values of GDM IADPSG criteria[24] were selected as controls after matching for age (±2 years) from the screened subjects.

Before recruiting for the study, written informed consent was taken from all the study participants. Individual demographics and information regarding current pregnancy, including past medical, obstetric, and family history, were collected with a structured interviewer-administered questionnaire. All pregnant women who tested positive for GDM were treated according to the standard guidelines with medical nutrition therapy and physical exercise with or without medical treatment.[25] At every monthly antenatal visit, glycaemic control was assessed in the GDM women and the therapy was titrated accordingly. All pregnant women with GDM were reassessed after 4 weeks postpartum with 75 g of OGTT (0 and 120 min) for the persistence of diabetes and were educated regarding lifestyle modifications for the prevention of T2DM in the future.

Study methods

A thorough clinical examination with the recording of height, weight, and BMI (weight/height^2) was performed. Body weight was measured using an Atlas electronic scale (range 400 g to 200 kg) to the nearest 0.1 kg. The subjects' height without footwear was measured to the nearest 0.1 cm using a wall-mounted stadiometer while standing straight and relaxed with light and minimal clothing, with the head in the Frankfurt plane. BMI was subsequently calculated as the ratio of weight in kilograms and square of height in meters. The eligible participants underwent 75-g OGTT test after an overnight fast for a minimum of 8 h. An intravenous cannula was placed in the antecubital vein of the forearm and was kept patent with regular flushing with normal saline. The first sample was taken immediately after cannulation and was marked as zero (0). Then, 75 g of oral anhydrous glucose dissolved in 200 mL of water was given to be drunk over 5–10 min. Subsequent sampling was performed at 30, 60, and 120 min after completing the glucose drink. Blood samples for estimation of glucose, insulin, glucagon, GLP-1, and GIP were collected. Women were instructed not to take any other food until the last sample was taken. A freshly prepared glass tube containing both a cocktail protease inhibitor (from Sigma-Aldrich Company) and Ethylenediamine tetraacetic acid (EDTA) was used to collect plasma to assess glucagon, GLP-1, and GIP, complying with the international guidelines for sample collection by Holst *et al*.[26] The cold chain was maintained throughout the sample collection time. Plain and sodium fluoride tubes were used to collect and estimate serum insulin and plasma glucose, respectively.

Assay methods

Plasma glucose and fasting lipid parameters were measured on the same day using the AU5800 analyzer (Beckman Coulter, CA, USA). Serum and plasma samples were stored at −80˚C for insulin, glucagon, GLP-1, and GIP assays. A chemiluminescence assay (ADVIA Centaur XP Immunoassay System, Siemens Healthcare Global, USA) was used to measure serum insulin levels.

Plasma glucagon was assayed using an R and D Systems enzyme-linked immunosorbent assay (ELISA) kit (Quantikine, Catalogue number DGCG0). The assay had < 12% cross-reactivity with oxyntomodulin and had no significant cross-reactivity with GIP, GLP-1, GLP-2, or glicentin-related pancreatic polypeptide

with a minimum detectable level of 2.12 pg/mL. The intra-assay CV for glucagon at 315 ± 10.3 pg/mL, 618 ± 22 pg/mL, and 1024 ± 27.6 pg/mL concentrations were 3.3%, 3.6%, and 2.7%, respectively. Its inter-assay CV at 354 ± 30.7 pg/mL, 653 ± 37.8 pg/mL, and 1080 ± 63.6 pg/mL concentrations were 8.7%, 5.8%, and 5.9%, respectively.

Plasma total GLP-1 was estimated using the ELISA kit from Merck Millipore (Catalogue number EZGLP1T-36K). The lower limit of detection sensitivity for the GLP-1 assay was 1.5 pM, with a range of approximately 4.1 to 1000 pM. It did not cross-react significantly with GLP-2, GIP, glucagon, or oxyntomodulin. Its intra-assay CV at mean GLP-1 concentrations of 32 and 216 pM was 1% and 2%, respectively, whereas the inter-assay CV was <12% and <10% at the mean GLP-1 concentrations of 39 and 220 pM, respectively.

Plasma total GIP was estimated by the ELISA kit from Merck, Millipore (Catalogue number EZHGIP-54K). The lower detection sensitivity limit for the GIP assay is 4.2 pg/nL. The assay did not cross-react significantly with GLP-1, GLP-2, glucagon, or oxyntomodulin. Its intra-assay CV at mean GIP concentrations of 15 and 185 pg/mL was 6.7% and 8.8%, respectively, whereas the inter-assay CV was 6.1% and 1.8% at the mean GIP concentrations of 26 and 166 pg/mL, respectively.

Calculation of indices

Insulin sensitivity was estimated by the homeostatic model assessment 2–insulin resistance (HOMA2-IR)[27] and the Matsuda index (MI). The MI measures whole-body insulin sensitivity and was calculated using the formula $MI = 10000/\sqrt{G_0 \times I_0 \times G_{mean} \times I_{mean}}$.[28] Insulin secretion was assessed by the homeostatic model assessment 2–beta (HOMA2-B), the insulinogenic index (IGI), and the oral disposition index (oDI). IGI is a measure of early-phase insulin response and is calculated as $IGI = \Delta I_{0-30}/\Delta G_{0-30}$.[29] The oDI was calculated using the formula $oDI = (\Delta I_{0-30}/\Delta G_{0-30}) \times (1/\text{fasting insulin})$, and it is a composite marker for both early insulin secretion and insulin sensitivity.[30] The insulin secretion sensitivity index-2 (ISSI-2), a measure of beta-cell function in relation to insulin sensitivity, was calculated using the formula $ISSI\text{-}2 = (AUC_{\text{insulin 0-120}}/AUC_{\text{glucose 0-120}}) \times MI$.[31] The linear trapezoidal rule calculated the total area under the curve (AUC) for glucose, insulin, glucagon, GLP-1, and GIP. Early glucagon suppression during the first 30 min after oral glucose administration was calculated by the formula glucagon suppression index $GSI_{30} = [1\text{-}(glucagon_{30}/glucagon_0)] \times 100\%$. Late glucagon suppression from 30 to 120 min was calculated using the formula $GSI_{30\text{-}120} = [1\text{-}(glucagon_{120}/glucagon_{30})] \times 100\%$, and overall glucagon suppression during the entire OGTT was calculated using the formula total $GSI = [1\text{-}(glucagon_{120}/glucagon_0)] \times 100\%$.[32]

Statistical analysis

The sample size was calculated using power and sample size software (version 3.1.2). The primary variables for assessing the outcome were MI and ISSI-2. The sample size was estimated to be 10 for MI (mean difference of 1.75, SD 1.16) and 20 for ISSI-2 (mean difference of 279, SD 21), respectively, in each group with 90% power and taking alpha as 5%.[33] Expecting a 20% dropout, the sample size was finalised as 25 in each group. Statistical analysis was performed using SPSS version 22. The distribution of the data was assessed using the Kolmogorov–Smirnov test. Normally distributed continuous variables are expressed as mean ± standard deviation (SD). Continuous variables that were not distributed normally are presented as median with an interquartile range (IQR). Categorical variables are represented by a percentage. Categorical variables were compared using Pearson's Chi-squared test or Fisher's exact test. In normally distributed data, the independent student *t*-test was used as a test of significance to compare between two groups. Similarly, the Mann–Whitney *U* test compared two groups when the data were non-normally distributed. Pearson's correlation test assessed the correlation between two normally distributed parameters, whereas Spearman's correlation test was used for non-normally distributed data. A *P* value of <0.05 was considered significant. All graphs were drawn using GraphPad Prism (version 9.2.0).

Ethical aspects

This prospective, observational study was carried out in the Department of Endocrinology of a tertiary care centre in South India from July 2018 to July 2020 after obtaining approval from Institute Ethics Committee (JIP/IEC/2018/0133, dated 07/08/2017) and was conducted according to the principles of the Helsinki Declaration II. Written informed consent was obtained for participation in the study and use of the patient data for research and educational purposes.

Results

A total of 110 pregnant women were screened for enrollment in the study. All underwent 75-g OGTT test at 24–28 weeks of gestation, and 25 women who fulfilled the diagnostic criteria of GDM as per the IADPSG criteria were enrolled as cases. Among the 85 NGT women, 25 were recruited after matching with cases for age (±2 years). A total of 50 women, 25 with GDM (cases) and 25 with NGT (controls) were finally recruited into the study [Figure 1]. Two NGT women withdrew their consent after sampling and hence were excluded from the analysis. The remaining 48 women were followed up until delivery. All GDM women received advice regarding diet and lifestyle interventions from trained personnel. Only one woman required additional insulin therapy as her blood glucose levels were not controlled by diet and lifestyle modification. None of the GDM women were initiated on any oral hypoglycemic agents. Amongst the GDM group, 17 women were re-evaluated with postpartum OGTT at a median of 12.5 weeks postpartum (IQR 10–14.5) for diabetes status. Amongst them, three had abnormal OGTT. One woman had a diagnosis of diabetes and two had prediabetes. Eight

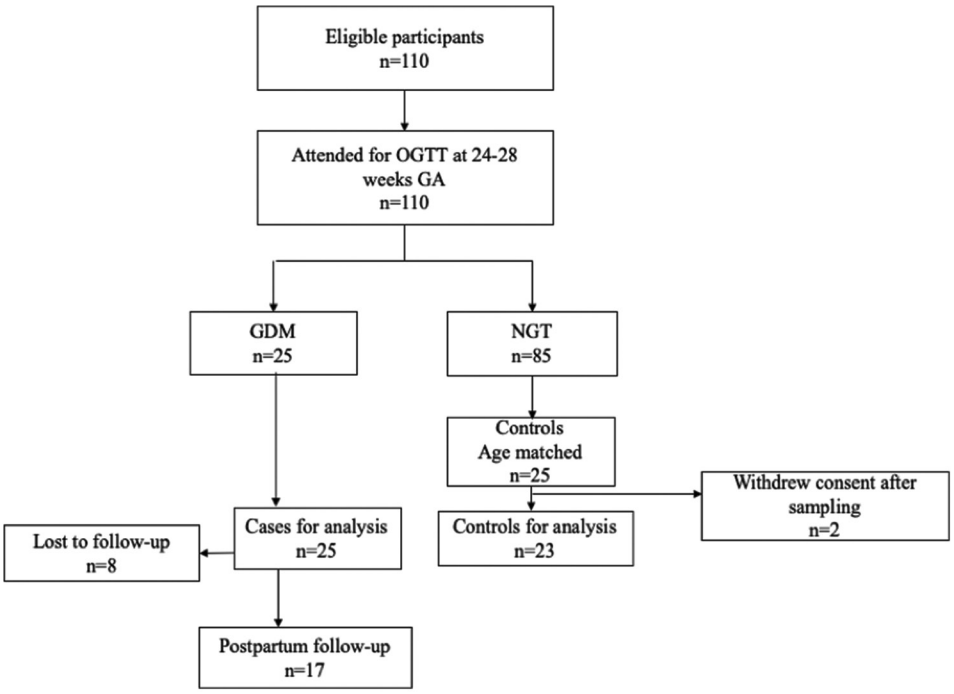

Figure 1: Flow diagram depicting recruitment of study participants

Table 1: Baseline characteristics and perinatal outcomes of the study population

Parameters	GDM (*n*=25)	NGT (*n*=23)	*P*
Age (years) (mean±SD)	25.7±3.4	24.2±3.5	0.14
GA (weeks) (mean±SD)	26.3±1.6	26.1±1.6	0.68
Gravida status (median, IQR)	1 (1-4)	2 (1-3)	0.26
Primi gravida (*n*, %)	10 (40%)	14 (61%)	0.14
H/O GDM in a previous pregnancy (*n*, %)	2 (8%)	0	
H/O PIH (*n*, %)	2 (8%)	1 (4%)	0.99
Hypothyroidism (*n*, %)	4 (16%)	3 (13%)	0.99
Family h/o T2DM (*n*, %)	7 (28%)	6 (26%)	0.99
BMI (Kg/m^2) (mean±SD)	23.4±3.7	22.4±4	0.38
SBP (mm Hg) (mean±SD)	109.2±13.2	107.5±10.8	0.63
DBP (mm Hg) (mean±SD)	69.0±8.2	69.1±6.7	0.98
TSH (μIU/mL) (mean±SD)	2.5±1.5	2.5±1.3	0.98
TG (mg/dL) (mean±SD)	190.1±46.3	175.6±50.5	0.37
GA (weeks) at delivery (mean±SD)	38.3±1.60	39±0.95	0.8
Birth weight (g, mean±SD)	2977±418	2895±349	0.47
LGA (*n*)	1	0	
SGA (*n*)	1	6	0.28
Congenital anomaly (*n*)	1*	0	
Perinatal asphyxia (*n*)	3	0	
NICU admission for ≥24 h (*n*)	3	1	0.61
Neonatal jaundice (*n*)	6	4	0.72

BMI- body mass index, DBP- diastolic blood pressure, DM-diabetes mellitus, GA- gestational age, GDM – gestational diabetes mellitus, IQR- interquartile range, LGA- large for gestational age, NGT- normal glucose tolerance, NICU- neonatal intensive care unit, PIH- pregnancy-induced hypertension, SBP- systolic blood pressure, SD- standard deviation, SGA- small for gestational age, TG- triglycerides, TSH- thyroid stimulating hormone. * Pierre Robin sequence anomaly

GDM women were lost to follow-up in the post-partum period, and hence their glycemic status postpartum could not be documented.

Baseline characteristics

The baseline characteristics of the study women, including mean age, gestational age (GA), gravida status, and BMI were

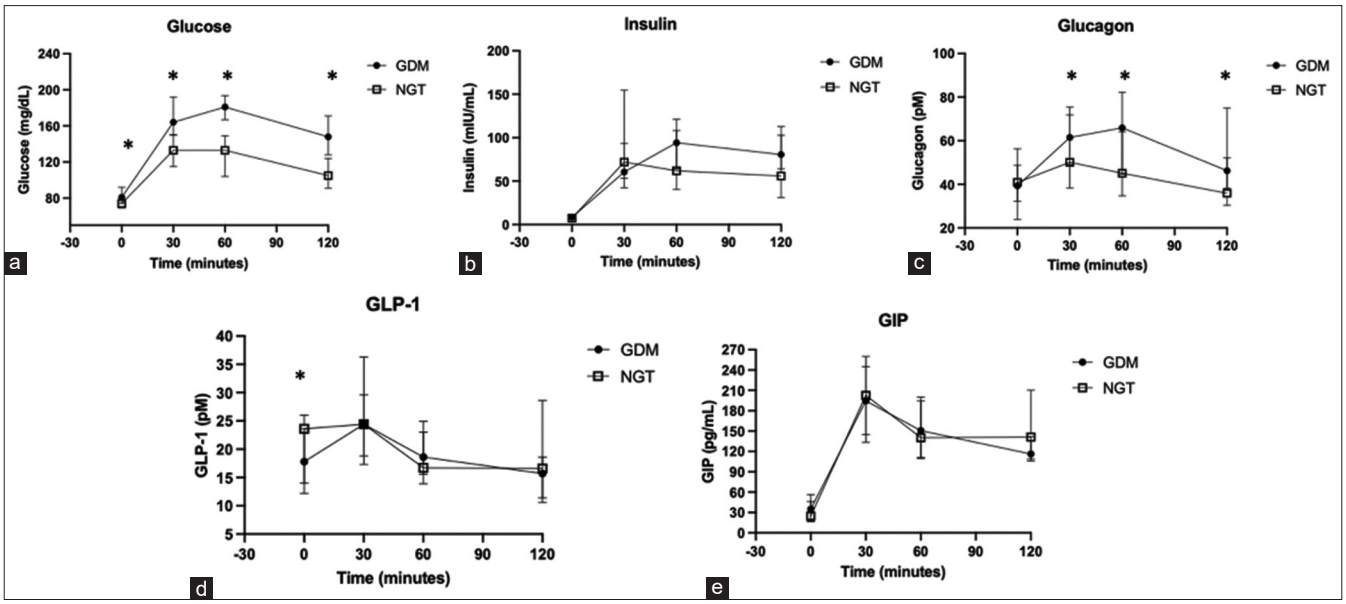

Figure 2: Line diagram comparing the excursion of different biochemical parameters among two groups during oral glucose tolerance test. (a) Glucose, (b) Insulin, (c) Glucagon, (d) Glucagon-like peptide-1 (GLP-1), (e) Glucose-dependant insulinotropic polypeptide (GIP)

Table 2: Glucose, insulin and GLP-1 levels, basal and after glucose ingestion

Parameters	GDM (*n*=25)	NGT (*n*=23)	*P*
Glucose (mg/dL) 0 min	83±10.5	75.1±7.8	<0.01
Glucose (mg/dL) 30 min	169.4±22.8	133.4±19.4	<0.01
Glucose (mg/dL) 60 min	185.8±26.6	128.9±28.9	<0.01
Glucose (mg/dL) 120 min	150.8±33	107.9±22.9	<0.01
Insulin (mIU/mL) 0 min	9.0±4.0	8.0±4.2	0.43
Insulin (mIU/mL) 30 min*	60.5 (42.4-93.4)	71.9 (53.3-154.6)	0.16
Insulin (mIU/mL) 60 min*	94.3 (63.7-121.2)	61.9 (40.3-108.2)	0.07
Insulin (mIU/mL) 120 min	87.5±35.5	76.7±61.1	0.45
AUC_{0-120} insulin (miU/mL min)*	7810 (6509-11759)	6463 (4685-11769)	0.23
AUC_{0-30} insulin (miU/mL min)*	1057 (746-1551)	1159 (900-2446)	0.20
GLP-1 (pM) 0 min	17.3±6.5	22.2±9.7	0.04
GLP-1 (pM) 30 min*	24.4 (18.8-29.6)	24.4 (17.3-36.3)	0.86
GLP-1 (pM) 60 min*	18.6 (15.6-23.0)	16.7 (13.9-24.9)	0.34
GLP-1 (pM) 120 min*	15.7 (10.6-18.6)	16.6 (11.4-28.6)	0.27
AUC_{0-120} GLP-1 (pMmin)*	2374.5 (1782.7-2770.5)	2344.5 (1818.0-3636.0)	0.81
AUC_{0-30} GLP-1 (pMmin)	652.2±244.9	744.3±309.8	0.26

Values are expressed in mean±SD, *Median with interquartile range. AUC- area under curve, GDM-gestational diabetes mellitus, GLP-1- glucagon-like peptide 1, NGT-normal glucose tolerance

comparable between the two groups [Table 1]. Seven and six women in the GDM and NGT groups had a family history of T2DM, respectively. The mean GA at delivery was 38.3 ± 1.60 and 39 ± 0.95 weeks (P = 0.80) for the GDM and NGT groups, respectively. The average birth weight was similar in both groups (GDM, 2977 ± 418 g, and NGT, 2895 ± 349 g, P = 0.47). Fetomaternal outcomes of the study population are presented in Table 1.

Change in insulin sensitivity and beta-cell function

Plasma glucose was significantly higher at all time points in the GDM group when compared to the NGT group [Figure 2a]. Insulin levels at all time points and $AUC_{insulin}$ were comparable in both groups [Table 2, Figure 2b]. There was no difference in HOMA2-IR between the groups, but the MI was lower in the GDM group as compared to the NGT group (P = 0.04). IGI, oDI, and ISSI-2 were significantly lower in the GDM group as compared to the NGT group. Though HOMA2-B was lower in the GDM group, the difference was not statistically significant. [Table 3]

Correlation of different parameters with insulin sensitivity and beta-cell function in GDM

Univariate analysis was performed to find predictors for insulin sensitivity and beta-cell dysfunction in GDM women. The ISSI-2 index showed a negative correlation

with BMI (r = -0.47, P = <0.01). Similarly, MI showed a negative correlation with diastolic blood pressure (r = -0.41, P = 0.04). [see supplementary Table 1]. Women with a history of hypothyroidism had significantly lower MI (2.9 vs. 4.7, P = 0.04). There was no significant difference in ISSI-2 or MI in GDM women with respect to a family history of T2DM, gender of baby, or gravida status (data not shown).

Glucagon excursion during OGTT

Fasting plasma glucagon was comparable between the two groups. Post-glucose load, plasma glucagon levels showed an initial mild increase at 30 min, followed by progressive suppression to reach levels below baseline at 120 min in NGT women. On the contrary, in GDM women, glucagon levels continued to increase until 60 min after the glucose load, following which the levels showed delayed mild suppression. However, the suppressed levels were above the baseline levels even at 120 min [Figure 2c]. Total $AUC_{glucagon}$ was significantly higher in the GDM group [Table 4]. To avoid the influence of the volatility in the levels of fasting plasma glucagon, we evaluated the glucagon responses using the change in the levels of glucagon (ΔGlucagon) from baseline (0 min) to each time point during the OGTT. ΔGlucagon levels at all time points were significantly higher in the GDM group. The early glucagon suppression index (GSI_{30}) was negative during the first 30 min post-glucose load, suggesting non-suppression of glucagon in both groups. However, late GSI during 30–120 min post-OGTT ($GSI_{30\text{-}120}$) was positive, suggesting a late glucagon suppression in both groups. GSI_{30} was significantly lower in the GDM group, suggesting a greater rise in plasma glucagon level during the first 30 min of OGTT in the GDM group, whereas $GSI_{30\text{-}120}$ was comparable between the two groups [Table 4].

Table 3: Difference in insulin resistance and beta-cell function indices between the two groups

Parameters	GDM (n=25)	NGT (n=23)	P
$HOMA_2IR$	1.14±0.53	0.99±0.51	0.34
Matsuda Index*	4.31 (3.03-5.49)	5.42 (4.08-8.71)	0.04
HOMA2B*	113.6 (95.9-134.5)	122.7 (104.5-149.1)	0.173
IGI*	0.64 (0.46-0.99)	1.59 (0.84-2.16)	<0.01
ISSI-2	1.99±0.73	3.18±0.91	<0.01
oDI*	0.08 (0.06-0.13)	0.19 (0.14-0.27)	<0.01

Values are expressed in mean±SD, *Median with interquartile range. GDM-gestational diabetes mellitus, HOMA2-IR- Homeostatic Model Assessment of Insulin Resistance – 2, IGI-insulinogenic index, ISSI-2- insulin secretion and sensitivity index-2, NGT-normal glucose tolerance, oDI- oral disposition index

Correlation between glucagon excursion with insulin sensitivity and beta-cell function

The glucagon-insulin ratio at 30 min (G/I_{30}) was higher in the GDM group compared to the NGT group [Table 4]. We could not find any correlation between glucagon levels at any time points or GSI with insulin sensitivity index MI. However, fasting glucagon levels and GSI_{30} in the GDM group had a negative correlation with ISSI-2 (r = -0.42, P = 0.04 and r = -0.55, $P \leq 0.01$ respectively) [Supplementary Table 1].

Correlation between incretin axis and islet cell function

GDM women had significantly lower fasting GLP-1 levels. However, GLP-1 levels at 30, 60, and 120 min post-glucose load and $AUC_{GLP\text{-}1}$ were comparable in both groups [Table 2, Figure 2d]. GIP levels at all time points and AUC_{GIP} were comparable in both groups [Supplementary Table 2, Figure 2e]. There was no correlation between the total $AUC_{GLP\text{-}1}$ and any beta-cell function indices such as ISSI-2 or oDI in the GDM group. However, the total $AUC_{GLP\text{-}1}$ had a significant correlation with ISSI-2 in the NGT group (r = 0.45, P = 0.03). Total $AUC_{GLP\text{-}1}$ showed a positive correlation with GSI (r = 0.47, P = 0.02) in the GDM group [Supplementary Table 3].

Table 4: Glucagon trends after glucose load in both groups

Parameters	GDM (n=25)	NGT (n=23)	P
GLG (pM)* 0 min	40.7±21.8	46.3±23.8	0.33
GLG (pM)* 30 min	68.2±26.1	58.9±30.5	0.04
GLG (pM)* 60 min	67.7±24.4	55.4±30.6	0.03
GLG (pM)* 120 min	57.0±23.1	45.4±23.3	0.01
ΔGLG (pM) 30 min	24.6 (5.2-38.5)	9.5 (4.0-19.4)	0.03
ΔGLG (pM) 60 min	30.4 (3.3-46.5)	2.6 (-0.5-15.3)	0.01
ΔGLG (pM) 120 min	10.8 (-7.0-42.6)	3.7 (-8.4-7.7)	0.04
$AUC_{0\text{-}120}$GLG (pM min)*	7411.7±2418.6	6320.1±3217.1	0.02
$AUC_{0\text{-}30}$ GLG (pM min)*	1633.5±598.4	1578.5±775.6	0.32
GSI_{30}	-62.6 (-203.3- -13.2)	-24.7 (-49.1- -7.6)	0.03
$GSI_{30\text{-}120}$	19.6 (-0.4-32.9)	20.9 (10.8-31.3)	0.59
Total GSI	-43.0 (-116.6-13.3)	-7.9 (-22.4-22.3)	0.03
G/I_0	5.2 (3.4-6.8)	5.9 (3.6-9.2)	0.24
G/I_{30}	1.1 (0.7-1.4)	0.7 (0.4-0.9)	0.01
G/I_{60}	0.7 (0.6-1.1)	0.7 (0.4-1.3)	0.86
G/I_{120}	0.6 (0.4-0.9)	0.8 (0.4-1.4)	0.61

Values are expressed in the median with interquartile range, *mean±SD. AUC-area under the curve, ΔGLG- delta glucagon, G- glucose, GDM- gestational diabetes mellitus, GLG- glucagon, GSI- glucagon suppression index, I-insulin, NGT- normal glucose tolerance

Discussion

This study evaluated insulin sensitivity, islet-cell function, and incretin axis in Asian-Indian GDM women and compared it with NGT pregnant women as controls. OGTT-based islet cell function and insulin sensitivity assessment tools are more physiological and can be paired with their routine screening test for GDM.[34] Insulin sensitivity indices derived using OGTT measure both hepatic and peripheral insulin sensitivities and have a better concordance with clamp-derived indices than the fasting sample-derived insulin sensitivity indices.[35]

We found no significant difference in HOMA2-IR ($P = 0.34$), a marker of hepatic insulin resistance between GDM and NGT women. However, the MI, which reflects the whole-body insulin sensitivity, was significantly low (median 4.31 vs. 5.42, $P = 0.04$) in GDM women. Approximately 80% of insulin-dependent glucose disposal occurs in the periphery in normal and diabetic individuals.[36] Therefore, the impaired insulin-mediated glucose disposal in pregnancy should also be reflected mainly by a greater impairment in peripheral than in hepatic insulin sensitivity.[36] This explains the similar hepatic insulin resistance in the two groups in our study.

A hyperbolic relationship exists between insulin sensitivity and insulin secretion. Previous studies have shown that the hyperbolic curve in women with GDM shifts down and to the left compared with that in women with NGT.[37] The disposition index, which is the product of insulin sensitivity and insulin secretion, measures early beta-cell function in relation to insulin sensitivity during OGTT.[30] We assessed the oDI, which was significantly low in women with GDM compared with NGT women. ISSI-2, another composite index of insulin secretion and insulin resistance, was also significantly low in the GDM group.

Fasting glucagon levels were comparable in both groups in our study. Post-glucose load, GDM women failed to suppress glucagon secretion. In the NGT group, there was a paradoxical rise in glucagon levels at 30 and 60 min, with late suppression of glucagon levels at 120 min. A few studies comparing plasma glucagon levels in GDM and healthy women have been published in the Caucasian population. Some found that GDM women had greater fasting plasma glucagon levels than healthy pregnant women.[17,38] Other studies have inconsistently shown similar plasma glucagon levels in both fasting and post-glucose challenge scenarios among women with GDM.[13,39] However, in most of these studies, plasma glucagon was measured with a radioimmunoassay that used polyclonal antibodies against the glucagon C-terminal region. Such assays might not accurately measure plasma glucagon because of cross-reactivity against other proglucagon fragments. Hence, we used a quantitative sandwich ELISA for glucagon estimation with almost nil cross-reactivity against other proglucagon fragments.

A recent study by Horie *et al*.[40] assessing glucagon response to 75-g OGTT test in pregnant GDM women showed a paradoxical augmentation of the early-phase glucagon secretion (0–30 min) after glucose load in the insulin-treated GDM group but not in the diet-controlled GDM women. They assessed a small number of NGT women who showed early physiological suppression of glucagon after the glucose load. Our study, however, showed negative GSI_{30} suggestive of non-suppressible early glucagon secretion after glucose load in NGT women. Similar to our study, Beis *et al*.,[17] in a study performed in Greece, had documented a late suppression of glucagon secretion after 120 min of OGTT in NGT women. As there are no previous studies on the Asian-Indian population, this unique trend of non-suppressed early glucagon response in NGT women may be unique to our ethnicity. Further studies are needed to establish this association.

Like glucose, insulin is also an important regulator of plasma glucagon concentration.[9] Insulin modulates glucagon secretion by reducing the sensitivity of potassium channels in pancreatic alpha cells.[41] Insulin resistance at the level of the pancreatic alpha cell and impaired insulin secretion due to beta-cell dysfunction contribute to impaired glucagon suppression observed in patients with T2DM.[42] In the current study, we found that glucagon levels negatively correlated with beta-cell function index ISSI-2. We could find no similar correlation with insulin resistance index MI. The lower beta-cell function and the lesser suppression of glucagon secretion explain the higher G/I_{30} ratio at 30 min in this study.

Our study found lower fasting GLP-1 levels in GDM women with no significant differences in GLP-1 levels post-glucose load in both groups. GIP levels were comparable in both groups at all time points. Mosavat *et al*.[12] similarly found lower fasting GLP-1 and GIP levels in GDM women when compared to NGT women. Bonde *et al*.[13] evaluated GLP-1 levels after a mixed meal in GDM women during the third trimester of pregnancy and 3 months postpartum. Fasting GLP-1 levels were lower in the GDM group during pregnancy and postpartum. Also, GDM women had significantly reduced postprandial GLP-1 response. This was in contrast to the studies by Cypryk[14] and Reyes- López *et al*.,[15] where higher fasting GLP-1 levels were observed in GDM women. Cypryk *et al*.[14] documented similar incremental GLP-1 response post-75-g OGTT test in GDM and NGT groups. GIP levels were also comparable at all times, as found in our study. O'Malley *et al*.[18] found no difference in fasting GLP-1 and GIP levels. Lencioni *et al*.[16] who assessed GLP-1 levels during a 100-g OGTT test in GDM and NGT women, found comparable AUC_{GLP-1} in both groups.

Incretin hormones GLP-1 and GIP have differential effects on beta and alpha cells. Both stimulate beta-cell insulin secretion. However, in the alpha cells, they have the opposite effect. GLP-1 inhibits glucagon secretion, likely via somatostatin-dependent paracrine signalling, whereas GIP stimulates glucagon secretion through direct mechanisms.[43] As observed in our study, beta-cell dysfunction and lack of early glucagon suppression may be due to the dysfunctional incretin axis. The current study found significantly low fasting GLP-1 levels in GDM women. AUC_{GLP-1} levels positively

correlated with both beta-cell function index ISSI-2 and GSI. These findings likely reiterate the pathogenic role of incretin axis dysfunction in the decreased beta-cell secretion and glucagon suppression observed in GDM women.

The main strength of our study is that our GDM cohort was equally comparable with healthy controls with respect to age, GA, gravida status, and BMI. We used OGTT-based indices such as MI and ISSI-2, which are well-validated in pregnancy and have a reasonable correlation with clamp studies.[44] We followed international guidelines for sample collection[26] and for performing assays for glucagon, GLP-1, and GIP. Ours is the first study that evaluated glucagon and incretin axes using OGTT-based indices in GDM women of the Asian-Indian population. Also, we used quantitative sandwich ELISA for glucagon estimation, which has almost nil cross-reactivity against other proglucagon fragments and has shown comparable results with liquid chromatography–mass spectrometry.[45,46]

Our study has a few limitations. We could not use the gold standard clamp studies. Also, we did not use a mixed meal test, which would have been a more physiological stimulus for assessing both islet-cell function and the incretin axis. We opted for OGTT as it was easier and could be clubbed with the routine OGTT performed for the diagnosis of GDM. We have not assessed other incretins such as GLP-2 or cholecystokinin in our study. Additionally, we could not reassess the study subjects in the postpartum period. The longitudinal evaluation of the assessed postpartum parameters would have helped us better understand the heterogeneous roles of insulin resistance, islet cell dysfunction, and incretin axis in the pathogenesis of GDM in the Asian-Indian population.

Conclusion

GDM women of Asian-Indian ethnicity have high insulin insensitivity, low beta-cell function, and non-suppressible glucagon secretion post-glucose load. Impaired beta-cell function likely contributes to impaired glucagon suppression in them. Incretin axis dysfunction also abets their islet cell dysfunction.

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Conflicts of interest

There are no conflicts of interest.

Author's Contribution

SK conceived the study idea, guided the study and revised the manuscript critically. NN performed the study, acquired the data, and drafted the manuscript with crucial analytical input and guidance of JS. AR and CM helped in performing the study, acquiring data and revising the manuscript. HS and BZ guided the study and revised the manuscript critically. DN revised the manuscript and put forward critical inputs. All authors have read and approved the final version of the manuscript.

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Supplementary Table 1: Correlation between Matsuda index and ISSI-2 with other variables in GDM mothers

Variable	Matsuda index Correlation coefficient (*r*)	*P*	ISSI-2 Correlation coefficient (*r*)	*P*
Age	-0.1	0.64	-0.18	0.39
GA	0.22	0.29	0.26	0.22
BMI	-0.16	0.44	-0.47	0.02
SBP	-0.38	0.07	-0.3	0.15
DBP	-0.41	0.04	-0.19	0.37
AST	0.01	0.98	-0.17	0.44
ALT	-0.24	0.28	-0.08	0.71
TC	-0.07	0.77	-0.17	0.64
TG	-0.06	0.79	-0.25	0.27
LDL	004	0.87	0.32	0.16
HDL	-0.15	0.5	0.12	0.58
VLDL	-0.06	0.8	-0.12	0.59
GLG_0	-0.15	0.48	-0.42	0.04
GSI_{30}	-0.18	0.39	-0.55	<0.01
Total GSI	-0.19	0.35	-0.38	0.06

ALT- alanine transaminase, AST- aspartate transaminase, BMI- body mass index, DBP- diastolic blood pressure, GLG- glucagon, GSI- glucagon suppression index, GA- gestational age, HDL- high-density lipoprotein cholesterol, ISSI-2- insulin secretion and sensitivity index 2, LDL- low-density lipoprotein cholesterol, SBP- systolic blood pressure, TC- total cholesterol, TG- triglyceride, TSH- thyroid-stimulating hormone, VLDL- very low-density lipoprotein cholesterol

Supplementary Table 2: GIP levels, basal and after glucose ingestion

Parameters	GDM [*n*=22]	NGT [*n*=21]	*P*
GIP (pg/mL) 0 min	34.9 (17.3-56.3)	24.8 (16.8-45.9)	0.51
GIP (pg/mL) 30 min	194.5 (133.5-260.0)	202.5 (144.8-245.2)	0.79
GIP (pg/mL) * 60 min	158.2±54.5	154.5±56.5	0.82
GIP (pg/mL) 120 min	116. (106.1-141.8)	141.1 (108.8-210.2)	0.13
AUC_{0-120}GIP* (pg/ml min)	17505.1±5637.8	18499.5±6992.8	0.61
AUC_{0-30}GIP * (pg/mL min)	3605.5±1274.9	3657.6±1382.4	0.89

Values expressed in median with interquartile range, *mean±SD. AUC- area under the curve, GDM-gestational diabetes mellitus, GIP- Glucose-dependent insulinotropic polypeptide, NGT-normal glucose tolerance

Supplementary Table 3: Correlation of AUC_{GLP-1} and islet-cell function indices

Islet-cell function indices	GDM		NGT	
	Correlation coefficient	*P*	Correlation coefficient	*P*
ISSI-2	-0.24	0.91	0.45	0.03
oDI	-0.16	0.46	0.30	0.16
IGI/HOMA2-IR	-0.11	0.60	0.31	0.15
Total GSI	0.47	0.02	0.26	0.24
GSI_{30-120}	0.43	0.03	0.39	0.07

AUC- area under the curve, GLP-1- glucagon-like peptide 1, GSI- glucagon suppression index, HOMA2-IR- Homeostatic Model Assessment of Insulin Resistance – 2, IGI- insulinogenic index, ISSI-2- insulin secretion and sensitivity index 2, oDI- oral disposition index