Original Article

Clinical and Biochemical Markers in Early Pregnancy for Prediction of Gestational Diabetes Mellitus

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Abstract

Introduction: Gestational Diabetes Mellitus (GDM) is associated with an increased risk of feto-maternal and neonatal complications. Many of these complications can be reduced or eliminated, if GDM can be predicted in early pregnancy. Current risk prediction models lack a strong predictive value. In this study, we aim to evaluate the early trimester maternal parameters for future prediction of GDM. **Methods:** In this prospective observational study, we screened 581 consecutive healthy women with singleton pregnancy for GDM during their first antenatal visit. After informed consent, fasting blood samples were collected and stored at −80°C. GDM was diagnosed as per IADPSG criteria. During prospective follow-up, a total of 55 patients developed GDM. A total of 110 age and BMI-matched controls were recruited for comparison. In all women, we measured the Oral Glucose Tolerance test with 75 gm anhydrous glucose, fasting insulin, HbA1c, hsCRP, uric acid, and lipid Profile. HOMA-IR, HOMA-β, and QUICKI were also assessed. **Results:** The GDM cohort had significantly higher median waist circumference, 2 hr plasma glucose, HbA1c, fasting insulin, HOMA-IR, hsCRP, uric acid, and serum triglyceride levels. Multiple regression analysis revealed HbA1c (OR 5.264; $P = 0.007$), 2 hr PPG (OR 1.026; $P = 0.035$), QUICKI (OR 1.057; $P = 0.016$), uric acid (OR 1.931; $P = 0.013$) and neutrophil: lymphocyte ratio (OR 1.545; $P = 0.008$) to be independently associated with GDM outcome with combined area under the curve (AUC) of 0.850, a sensitivity of 72.7%, and a specificity of 87.3%. **Conclusion:** Fasting Insulin, HbA1c, HOMA-IR, hsCRP, and Uric acid levels are significantly increased in early pregnancy in individuals who subsequently develop GDM.

Keywords: Clinical prediction model, early trimester, gestational diabetes mellitus

Introduction

Gestational Diabetes Mellitus (GDM) as per the World Health Organization (WHO) is defined as any degree of glucose intolerance with its onset or first diagnosis during pregnancy.[1] Asian women have shown a high predilection for GDM development compared to their Western counterparts. It has been reported that Indian women have an 11-fold higher risk of developing GDM.[2] There is a rise in the prevalence of T2DM, obesity, as well as GDM.[3] It is a well-known fact that GDM is associated with multiple adverse feto-maternal outcomes.

Among 5–10% of cases, hyperglycemia persists immediately beyond pregnancy. However, there is a 10-fold higher risk of development of future T2DM among GDM women within 10-20 years.[4] There is an 8-fold higher risk of DM development among the offspring of GDM women.[5] Thus GDM with poorly controlled glycemic status has both short and long-term adverse consequences. Screening for GDM based only on risk factors has poor yield (diagnostic rate ~ 60%) with a 30–40% false negative rate.[6] Thus, this suggests that additional unrecognized factors may contribute to this higher risk of GDM development. Despite extensive research, the optimal method for identifying high-risk GDM women in the early stages of pregnancy remains elusive. Multiple risk factor assessment gives better prediction than a single risk factor for the development of GDM.[7]

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No preferred international consensus or testing approach is available presently. Any improvement in this approach should ideally be pragmatic, especially in resource-poor settings. Therefore, this study was undertaken to study the utility of various clinical and biochemical parameters in early pregnancy for the prediction of the future development of GDM.

Materials and Methods

We performed a prospective observational study in the early gestational period (<14 weeks) to predict the future development of GDM. All pregnant mothers attending our hospital for antenatal care in the early trimester, qualifying the inclusion criteria were enrolled. An ultrasound scan was used to confirm gestational age. Our Study included all pregnant women aged >18 years with singleton pregnancy at the first antenatal visit with <14 weeks gestational age and no known history of thyroid disease or diabetes. All those pregnant women who had multiple pregnancies, IVF conception, were diagnosed with diabetes mellitus before the current pregnancy, history of pancreatic disease, PCOS, or GDM or if on any medication that may lead to hyperglycemia were excluded from the study. Patients having hyperglycemia (FPG >92; 1 hr >180; 2 hr >153 or HbA1c >6.5%) on initial evaluation, history of chronic illness, and non-consenting individuals were also excluded,

The patient's information such as age, cigarette smoking, and alcohol use, underlying illnesses, history of GDM or macrosomia or stillborn child, parity, and obstetric and family history of diabetes were recorded in a proforma. Various maternal anthropometric parameters including weight and height, waist circumference (WC), and neck circumference (NC) were measured, and BMI was calculated as weight in kilograms divided by the square of height in meters.

Maternal fasting plasma samples were collected and stored at −80°C for subsequent biochemical analyses. All Samples were thawed to 30°C before running the assays. 75 grams glucose (or 82.5 grams monohydrate glucose) Oral Glucose Tolerance Test (OGTT) was done at the first visit. Patients who had normal OGTT values at the initial visit were enrolled in the study. All women underwent routine second-trimester screening for GDM (24–28 weeks' gestation) according to the IADPSG criteria.[8] Those women who did not follow within 24–28 weeks were screened for GDM whenever they visited after 28 weeks.

The participants were considered to have GDM if one or more of the following criteria was met; fasting plasma glucose levels: fasting, ≥92 mg/dl; 1-hour post glucose plasma glucose (PGPG) ≥180 mg/dl; and 2-hour PGPG ≥153 mg/dl. The participants were assigned to a GDM or normal control group, based on the results.

Anthropometry: Waist Circumference (WC) was measured using plastic tape in centimeters to the nearest 1 mm. Measurement was taken horizontally at the midpoint between the iliac crest and coastal margin in the mid-axillary line. The subject was standing erect and measurements were taken at the end of expiration.[9] Neck Circumference (NC) was measured in the midway of the neck, between the mid-cervical spine and mid-anterior neck, using a non-stretchable plastic tape with the subjects standing upright. We asked the subject to look straight ahead (head positioned along the Frankfurt plane), with shoulders down but not hunched while taking this reading, and cared not to involve the shoulder/neck muscles (trapezius) in the measurement.[9]

The biochemical parameters were carried out at our certified labs according to the standards of laboratory methods. The Insulin was measured using Cobas e 411 analyzer (Roche Diagnostics) with intra- and interassay variability was 3.2% and 4.2%, respectively. HOMA-IR was computed using the formula, HOMA-IR = [fasting glucose (mg/dl) × fasting insulin]/405. Beta cell function was assessed by HOMA-β using the equation: HOMA-β = (20 × FI [μU/mL]/(FBG [mmol/L] – 3.5)).[10] QUICKI was estimated by using the equation, QUICKI = [1/(log fasting Insulin + log fasting glucose)].[11] The uricase calorimetric method on the COBAS 6000 Plus machine was used for uric acid estimation. It had a coefficient of variance of 0.9%. HsCRP was measured by the Nelphometry method on the Siemen's Nephelometer analyzer. The normal range of the test value was between 0.01 and 0.1 mg/dL (0.1 –1 mg/L). The intra-assay and inter-assay CV was 0.59% and 1.97% respectively. Roche COBAS 6000 machine was utilized to measure HbA1c (HPLC method) according to NGSP/DCCT trial with intra- and inter-assay coefficients of variation was 0.78% and 1.68%, respectively. Lipid Profile was measured by enzymatic and Point method on COBAS 6000 Plus analyzer and LDL-cholesterol levels were calculated using the Friedewald formula. The Triglyceride Index (TyG index) was calculated using the following formula: Ln (Fasting triglycerides [mg/dL] × Fasting glucose [mg/dL]/2).[12]

Statistical analysis

Normally distributed variables (Using the Shapiro–Wilk test) were represented by the mean ± standard deviation (SD) and others by median (interquartile range). For continuous variables, the student's 't' test was used for parametric and the Mann–Whitney U test for non-parametric data. The categorical variables were compared by Chi-square test or Fisher's Exact test. The controls were selected in the ratio of 2:1 with GDM subjects after matching according to age and BMI using online MedCalc software. Univariate analysis computed significant variables to determine the odds ratio. The significant biochemical markers in univariate were analyzed in the binary-logistic regression model. The receiver operating characteristic (ROC) curve was used to find out the cut-off point of the significant variables. The odds ratio with a 95% confidence interval was calculated for the significant variable. For the difference to be considered statistically significant, a *P* value of less than 0.05 was considered. Microsoft Excel

and SPSS (version 28.0) were used for analyzing the data and creating bar graphs.

Ethical aspects

Institutional Ethics Committee - Institute of Medical Sciences and SUM Hospital [DMR/IMS.SH/SOA/2021031] was obtained and all the procedures performed in the study were in accordance with the ethical standards of the Ethical Committee and with the Helsinki Declaration of 1964 and its later amendments or comparable ethical standards. All participants gave the written informed consent.

Results

A total of 581 subjects were screened for the study. Of these subjects, based on exclusion criteria, 146 subjects were excluded and 101 refused study consent. The remaining 334 subjects were recruited and had their blood drawn at the first ante-natal visit. Out of it, 43 of the participants did not follow up for the 24–28 weeks OGTT. A total of 291 subjects turned up for repeat OGTT and were followed till delivery. Among them, 55 screened positive for GDM as per IADPSG criteria while 236 screened negative. Out of 236 control patients, using MedCalc statistical software, 110 controls were matched with the GDM group based on age and BMI parameters (1:2 ratio). The above findings are summarized in Figure 1.

As reported, both GDM and non-GDM controls were age and BMI-matched. The GDM group had a significantly higher proportion of subjects with a positive family history of Diabetes. No significant difference was noted between the two groups with regard to bad obstetric history, parity, hypertension, alcohol, or smoking. We noted that the presence of acanthosis nigricans was significantly higher in the GDM cohort compared to non-GDM controls (61.8% vs 24.5%; $P < 0.001$). The anthropometric measures like waist circumference (WC), neck circumference (NC), WC/Ht. the ratio was significantly higher in the GDM than in the non-GDM group ($P = 0.009$, 0.029, and 0.002, respectively). Ht/NC ratio was the significantly lower ratio in the GDM group ($P = 0.003$) [Table 1].

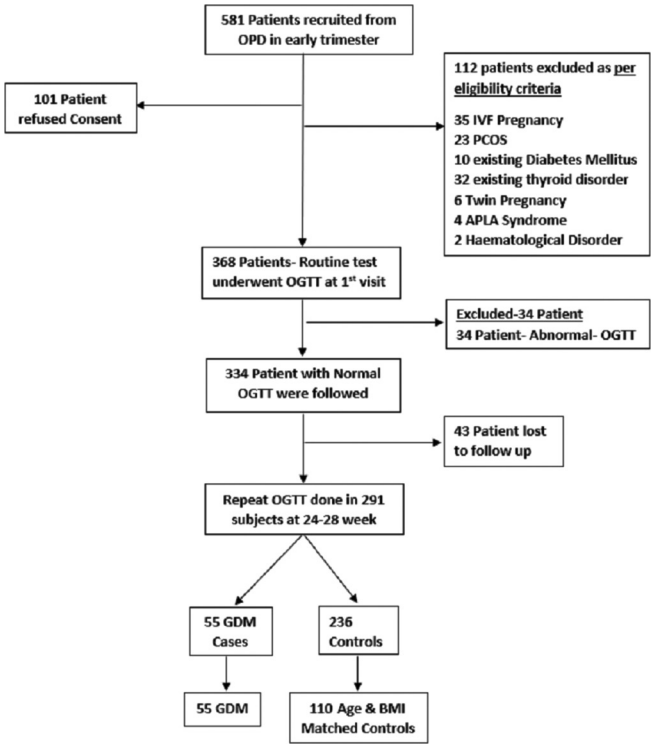

Figure 1: Flow Chart summarizing study design and recruitment

Various glycemic parameters like FPG, 1 hr PGPG, 2 hr PGPG, and fasting Insulin were compared between the two groups. The comparison of mean/median values of glycemic parameters is presented in Table 2. All the above glycemic parameters were significantly higher in the GDM group ($P < 0.05$). The mean HbA1c was significantly elevated in the GDM cohort ($P < 0.001$). When the two groups were stratified based on fasting insulin levels (IQR), it was noted that the majority of the GDM group (45.45%) had fasting insulin levels in the highest quartile in contrast to the majority of non-GDM controls had fasting insulin levels in the lowest quartile (33.6%) [Supplementary Figure 1].

GDM cohort had statistically significantly elevated inflammatory markers like hsCRP and uric acid ($P < 0.001$) [Table 2]. A significantly higher proportion of subjects in the control group had lower uric acid levels compared to the GDM group (53.6% vs 25.45%) whereas a higher proportion of the GDM cohort had raised hsCRP levels (89.1% vs 72.7%).

The parameters of insulin resistance like HOMA-IR were significantly higher in the GDM group compared to the non-GDM control ($P < 0.001$). Whereas, insulin sensitivity parameters like QUICKI were significantly lower in the GDM cohort. We also stratified the groups based on insulin resistance as mild, moderate, and severe as per HOMA-IR values. Among the GDM cohort, the proportion of subjects belonging to moderately and severely raised HOMA IR groups were 14.5% and 12.7%, respectively. This was significantly higher than the control population ($P < 0.001$) [Supplementary Figure 2].

The median triglyceride levels were higher in the women who subsequently developed GDM compared to those women who remained normoglycemic during follow-up (141 mg/dl vs 125.35 mg/dl), however, it was not statistically significant. Other lipid parameters like Total cholesterol, High-Density Lipoprotein cholesterol, Low-Density Lipoprotein cholesterol, very low-density lipoprotein cholesterol, non-HDL cholesterol, or LDL/HDL ratio were comparable between the groups without any statistically significant difference ($P > 0.05$). The triglyceride index (TyG index) is an emerging biomarker for the assessment of insulin resistance. When compared to controls, the GDM group had a significantly higher TyG index ($P = 0.01$) [Table 2].

Multiple logistic regression model for predicting GDM

The ROC curve analyses performed on each of the significant maternal variables for the prediction of GDM are depicted in Table 3. QUICKI had the highest AUC of

Table 1: Comparison of Demographic parameters between GDM and non-GDM control Groups

Demographic Profile	GDM Group (*n*=55)	Non-GDM Control Group (*n*=110)	*P*
Age	28 (24–30)	27 (25-29.25)	0.367[#]
Height (cms)	153.8±5.7	155.2±6.5	0.179*
Weight (in kg)	60.0±9.1	59.1±11.1	0.606*
BMI (kg/m^2)	25.3±3.6	24.5±4.0	0.168*
Family History of DM *n* (%)	30 (54.5)	42 (38.18)	0.045[$]
Bad obstetric History *n* (%)	20 (36.36)	26 (23.63)	0.099[$]
Parity			
Primigravida *n* (%)	27 (49.09)	68 (61.82)	0.135[$]
Multigravida *n* (%)	28 (50.91)	42 (38.18)	
H/O HTN *n* (%)	1 (1.8)	0	0.333[$]
H/O macrosomia *n* (%)	1 (1.8)	0	0.333[$]
WC (cms)	95.1±12.4	90.1±11.0	0.009*
NC (cms)	32.5 (30.5–33.5)	31.4 (30.0-33.1)	0.029[#]
WC/Ht Ratio	0.62±0.08	0.58±0.07	0.002*
Ht/NC Ratio	4.75±0.39	4.94±0.37	0.003*
Acanthosis Nigricans *n* (%)	34 (61.82)	27 (24.55)	<0.001[$]

[#]Mann–Whitney *U P*; *Independent samples *t*-test; [$]Fisher's Exact Chi square test. *n* - Number; H/O- History of; HTN – Hypertension; DM – Diabetes Mellitus; WC – Waist Circumference; NC – Neck Circumference; Ht – Height

Table 2: Comparison of Baseline Laboratory parameters between GDM and Non-GDM controls

Lab Parameters	GDM Group (*n*=55)	Non-GDM Control Group (*n*=110)	*P*
Hb (gm/dl)	11.90 (11.20–12.50)	11.90 (11.00–12.50)	0.693[#]
WBC	10.06 (9.21–11.10)	9.22 (7.58–10.21)	0.001[#]
Neutrophil	70.98±8.06	69.03±6.28	0.090*
Lymphocyte	22.68±7.07	24.22±5.98	0.144*
N/L Ratio	3.57±1.56	3.11±1.13	0.015*
Uric Acid (mg/dl)	3.70 (3.10–4.20)	3.00 (2.60–3.50)	<0.001[#]
hsCRP (mg/dl)	0.46 (0.31–0.77)	0.27 (0.1–0.47)	<0.001[#]
FPG (mg/dl)	84 (81.00–88.00)	83.00 (76.9.00–87.00)	0.031[#]
1 hr PPPG (mg/dl)	142.00 (138.00–151.00)	134.00 (116.92–150.25)	0.005[#]
2 hr PPPG (mg/dl)	117.69±14.65	105.52±19.45	<0.001*
Fasting Insulin (µU/mL)	12.42 (8.70–17.57)	7.50 (4.79–11.50)	<0.001[#]
HbA1c (%)	5.46±0.40	5.12±0.37	<0.001*
HOMA-IR	2.70 (1.70–3.70)	1.54 (0.92–2.24)	<0.001
HOMA-beta	209.70 (155.10–308.10)	143.53 (105.34–208.56)	<0.001
QUICKI	0.30 (0.30–0.40)	0.36 (0.34–0.39)	<0.001
Triglyceride (mg/dl)	141.00 (96.00–215.00)	125.35 (96.75–172.00)	0.128[#]
Total Cholesterol (mg/dl)	204.00 (174.00–233.00)	205.00 (168.00–243.00)	0.981[#]
HDL (mg/dl)	48.00 (40.00–57.00)	49.00 (41.00–56.00)	0.870[#]
LDL (mg/dl)	120.00 (98.00–146.00)	127.00 (105.25–153.25)	0.241[#]
Non-HDL (mg/dl)	149.00 (126.00–187.00)	151.50 (126.75–190.00)	0.993[#]
Triglyceride Index	8.73±0.58	8.53±0.41	0.01*

*Independent Samples '*t*' test; *P*. Data represented by Mean±SD. [#]Mann–Whitney *U*; *P*. Data represented by median (IQR). Hb – Hemoglobin, WBC – White Blood Cells, N/L – Neutrophil: Lymphocyte, RBC – Red blood cells. FPG – Fasting plasma glucose, PPPG – Post Prandial Plasma Glucose. Triglyceride index is calculated as: normal log [fasting triglycerides × fasting glucose (in mg/dL)]/^{2}. HDL – High-Density Lipoprotein, LDL – Low-density lipoprotein, TC- Total cholesterol

0.768 with sensitivity of 74.5% and specificity of 97.27%. The Triglyceride Index (TyG index) had a threshold of 9.16 which showed borderline significance ($P = 0.04$). The Youden Index was used to derive the cut-off of various glycemic parameters. Multiple forward stepwise logistic regression model was attempted to identify the independent factors for predicting GDM among pregnant women from significant biochemical parameters obtained in univariate analysis. The percentage of correct prediction was 89.1% for the control group and 60% for the GDM group. The significant parameters after multiple binary regression for GDM prediction are depicted in Table 4. In this model, the area under the curve was 0.85, 95% CI: 0.788 – 0.911 with $P < 0.001$. Utilizing the five parameters (Hba1c, 2hr PPG,

QUICKI, uric acid, and N/L ratio) derived from multiple regression analysis, the sensitivity, specificity, and diagnostic accuracy for GDM prediction were 72.7%, 87.3%, and 82.43%, respectively [Figure 2].

Discussion

GDM usually develops between the 24th and the 28th weeks of gestation and these women are at higher risk of maternal and neonatal complications. The prevalence of GDM is rising at rapid rate in developing countries. Hence there is a recommendation for universal screening at first visit. But still there are no suitable early screening method available for GDM detection. Different criteria for diagnosis of GDM used in different regions make it more difficult.

Recently multiple studies have been undertaken across the globe focusing on the prediction of GDM based on the maternal clinical parameters and novel biomarkers during the first trimester of pregnancy.[13] Various clinical risk-based prediction models designed for GDM prediction have moderate detection rates. The model based on family history, ethnicity, previous history of GDM, or BMI showed an ROC of 0.77 (95% CI 0.69–0.85).[14] In another prospective study involving a cohort of 7929 pregnant women, a predictive model was formed using similar clinical parameters that resulted in specificity, sensitivity, and AUC of 81%, 73%, and 0.824 (0.793–0.855), respectively for identifying GDM requiring insulin.[15] The addition of relevant bio-markers over the clinical risk factors will further enhance the prediction rate for GDM.

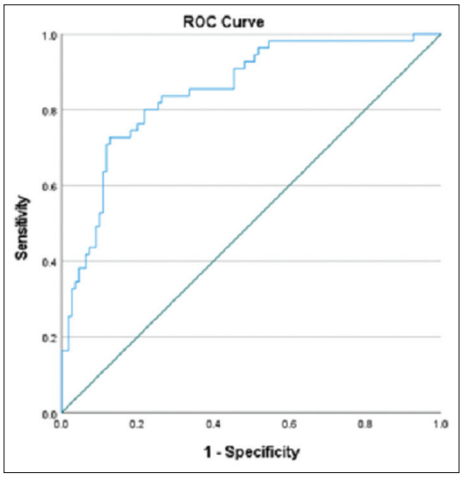

Figure 2: ROC curve analysis of 5 parameters (HbA1c, Uric Acid, QUICKI, 2 hr Post Plasma Glucose and N:L Ratio) for predicting Gestational Diabetes in first trimester

To date, multiple studies in the literature have evaluated the role of anthropometric parameters in pregnant women in relation to the birth outcome, especially birth weight. However, only a few of these have looked for early pregnancy or pre-pregnancy anthropometric parameters as a predictor for the onset of GDM.[8,14-16] Waist and neck circumference are important markers of obesity. Many studies have looked at waist circumference for pregnancy-related outcomes. In a pilot study by Madhavan *et al*.[16] conducted among Indian pregnant women during the 1st trimester of pregnancy found that a waist circumference cut-off of 85.5 cm and BMI cut-off of ≥24.3 kg/m^2 had the best prediction for GDM. A recent analysis showed that the cut-off for WC of 100 cm had 84% sensitivity and 70.9% specificity for GDM in the Turkish population.[17]

In our study, an optimal cut-off for a waist circumference of 91.09 cm had 67.3% sensitivity and 61.4% specificity for GDM. This cut-off is higher than the one proposed by WHO of 80 cm for Indian women. When WC of ≥100 cm was taken as a cut-off the specificity for GDM detection increased to 88.6%. We found that higher NC was significantly associated with GDM development (OD 1.199; 95% CI 1.058 – 1.359; $P = 0.004$). A cut-off of 32.23 cm had sensitivity and specificity of 61.8% and 66.5% respectively. Similar to our findings, a study of Chinese pregnant women (n = 97 GDM) found that NC has an OR of 1.29 for GDM with a cut-off of 33.8 cm.[18] Low height in a pregnant woman is associated with higher GDM rates and adverse perinatal outcomes.[19] In our study population, we observed that both our groups were comparable with regard to height.

To the best of our knowledge, the utility of Height–Neck circumference ratio (Ht:NC) has never been assessed as a GDM predictor. We found that non-GDM controls had higher Ht: NC ratios (4.94 ± 0.37 vs 4.75 ± 0.39). The computed ROC was 0.665 ($P < 0.001$; 95%CI 0.583 – 0.746) with a cut-off of 4.86 having sensitivity and specificity of 61.4% and 67.3% respectively. WC: Ht ratio showed good prediction for GDM

Table 3: ROC analysis of significant parameters for prediction of GDM

Particular	HbA1c	2 h PPG	Insulin	Uric Acid	HsCRP	TG Index	QUICKI	N:L Ratio
Cut Off Point	5.15	115.55	9.435	3.55	0.311	9.16	≤0.3	3.81
AUC	0.74	0.694	0.748	0.718	0.689	0.594	0.768	0.579
95%CI	0.66-0.82	0.61-0.78	0.67-0.83	0.64-0.80	0.61-0.77	0.49-0.69	0.69-0.83	0.48-0.68
Significance level *P* (area=0.5)	<0.001	<0.001	<0.001	<0.001	<0.001	0.049	<0.001	0.09
Sensitivity	83.60%	65.50%	70.90%	58.20%	76.40%	23.60%	74.55	41.8
Specificity	55.50%	73.60%	68.20%	76.4%	60%	95.50%	97.27	80.9
Positive Predictive Value	48.44%	55.37%	52.71%	55.22%	48.85%	72.36%	93.18%	72.59%
Negative Predictive Value	87.13%	81.01%	82.42%	78.52%	83.57%	71.46%	88.43%	99.76%
Diagnostic Accuracy	64.87%	70.90%	69.10%	70.33%	65.47%	71.56%	89.70%	80.77

Table 4: Multiple binary logistic regression model for GDM

Step	Variables	Wald Chi-square	*P*	Adjusted odds ratio (95% CI)
5	N/L Ratio	7.089	0.008	1.545 (1.122–2.128)
	HbA1c	7.395	0.007	5.264 (1.590–17.428)
	2 hr PPPG	4.447	0.035	1.026 (1.002–1.050)
	QUICKI	5.799	0.016	1.057 (0.00–0.038)
	S. Uric acid	6.122	0.013	1.931 (1.147–3.252)

with a cut-off of 0.62 [OR-4.790 (95% CI 2.564 – 8.950; $P < 0.001$)].

Many studies have suggested that higher uric acid levels in early pregnancy are related to increased risk of GDM development and thus it was opined that uric acid could serve as a future marker of GDM prediction.[20] We calculated the receiver operator characteristic analysis which gave a cut-off value of 3.39 mg/dl. A similar cut-off of 3.6 mg/dl showed a 3-fold higher risk of future GDM onset independent of BMI.[20] In the present study, median hsCRP levels were statistically significantly higher in the GDM vs non-GDM group (0.46 vs 0.27 mg/dl) with 89.1% of the GDM subjects having hsCRP levels above the normal range compared to 73% of the control group ($P = 0.017$). ROC analysis showed that hsCRP had the highest sensitivity in detecting GDM (72.7%) with a cut-off value of 0.24 mg/dl. Value above this threshold had a 3-fold higher risk of developing GDM (OR 3.391). Pregnancy is a subclinical inflammatory condition having higher hsCRP values and more so with higher BMI. hsCRP interferes with the post-receptor insulin signaling probably by upregulating the pro-inflammatory cytokine production like TNF-a or IL-6 leading to an insulin resistance state. Few studies have shown good sensitivity and specificity of hsCRP for predicting GDM.[21] Like CRP, N/L ratio is too considered a marker of chronic low-grade inflammation. In our prospective study design, we found that the N/L ratio in early pregnancy stages was significantly higher in the future GDM group. Even after multiple binary logistic regression analyses, the N/L ratio was significantly higher in cohorts with future GDM. Further large randomized prospective studies are needed to have a definite association of NLR as a GDM developmental marker.

Multiple studies have reported fasting, 1 hr post glucose, or 2 hr post glucose as a predictive marker for GDM and pregnancy outcomes but with poor specificity. A recent analysis of the Scandinavian population using WHO1999, IADPSG 2010, WHO2013, and Norway 2017 criteria for GDM diagnosis concluded that the sensitivity of FPG for GDM prediction varied between 70-75% using different criteria of diagnosing GDM and positive predictive value of 20-30%.[22] In recent combined analysis review for FPG revealed a linear relationship between first-trimester FPG and later-onset GDM risk. The addition of other parameters or biomarkers will further enhance the prediction accuracy. Phaloprakarn *et al*.[23] analyzed 193 pregnant women with 100 gm glucose using NDDG criteria (39 developed GDM) and found that that 1 hr post glucose value has the best diagnostic performance amongst the 4 glucose values for GDM prediction. A cut-off of ≥155 mg/dl was formulated with good sensitivity and NPV of 89.7% and 96.1% respectively with 64.3% specificity. In the present study, 1-hour post glucose plasma glucose (PGPG) turned out to be a better marker than FPG for GDM prediction with good sensitivity of 80% but low specificity using 136.5 mg/dl as a cut-off. A 2 hr PGPG value cut-off of 115.5 mg/dl was an independent predictor of GDM after multiple regression analysis. The patients with 2 hr PGPG >115.5 mg/dl had nearly 5 times higher chances of GDM development.

In our study, we observed that HbA1c is significantly raised in the GDM group. Early trimester HbA1c independently predicted the subsequent onset of GDM. HbA1c cut-off value of 5.15% had a high sensitivity of 78.2% and moderate specificity depicting a 4.4 times higher risk for subsequent GDM development. How useful is HbA1c is still a question of debate as normal ranges are not defined in pregnancy, unlike non-pregnant individuals. A retrospective study has indicated HbA1c cut-off of <4.8% excludes GDM with 95% sensitivity while another study limits this cut-off to <5.5%.[24]

We also found that fasting Insulin in early gestational weeks was an independent predictor for GDM (OR-1.092). A derived cut-off value of 9.4 micro-IU/mL had 70.9% sensitivity and 68.2% specificity. This cut-off for Insulin conferred five times higher risk for subsequent GDM development. Similar to our study, Correa *et al*.[25] also observed higher insulin levels with a median difference of 5 mIU/ml in the later onset GDM cohort compared to controls (7.5 µU/mL vs. 12.25 µU/mL, $P = 0.003$).

Our study revealed QUICKI as the best insulin index for GDM prediction over HOMA IR with significantly lower values observed in the GDM group. Veronica Falcone *et al*.[26] found that QUICKI was significantly lower in the early gestational period between 6 and 12 weeks in the group who later developed GDM. Normally insulin sensitivity improves in early gestation and then there is a progressive rise in resistance. However, in women who develop GDM at 24 weeks, insulin sensitivity is relatively lower at the early stages too compared to normoglycemic tolerance women.

Triglyceride Index (TyG) has gained importance in recent times as a marker of insulin resistance. TyG is a highly sensitive surrogate marker to detect insulin resistance. In the Brazilian population, TyG has emerged as a better marker than HOMA-IR to detect insulin resistance.[27] Very few studies have been carried out in relation to TyG and GDM risk. Song *et al*.[28] have reported that TyG index can predict GDM with OR 2.52 and this further increases in the Asian cohort to 3.30 ($P = 0.003$). In our study, we found we found that TyG index was significantly elevated in our GDM cohort with a mean difference of 0.2 ($P = 0.01$). TyG value of 9.1 had a very high specificity of 95.5% for GDM. A different

cut-off in our population may be owing to different ethnicity and dietary patterns.

After multiple regression analyses, HbA1c, 2 hr PPG, QUICKI, uric acid, and N/L ratio independently predicted the development of GDM. This result is similar to various reviews and meta-analyses that have highlighted the predictive role of these biomarkers.[25,29] Nanda *et al*.[30] combined maternal characteristics like age, BMI, family history of DM, and ethnicity with biochemical parameters like adiponectin and sex-hormone binding globulin to achieve AUC of 0.86 and 0.84 respectively to predict GDM. Our combined model had an AUC of >0.82 with a high sensitivity of 86%. These results also suggest that although hsCRP, and anthropometric measures like waist circumference are important independent predictors of GDM, they do not have significant predictive capabilities when interacting with other independent variables.

The study strengths include the utilization of multiple clinical and biochemical parameters for predicting GDM which are inexpensive, stable, easily measurable with automated instruments, and non-time consuming. The prospective nature of the study and a reasonably good cohort size are also the strengths of the study. The limitation of this study is the small number of GDM subjects obtained during follow-up. Our population consisted mainly of eastern Indian ethnicity and the generalizability of the model needs to be checked in other populations.

Conclusion

The results of our study establish the utility of commonly used and relatively inexpensive clinical and biochemical parameters in the early trimester of pregnancy for the subsequent development of GDM. These would be very helpful in resource-poor settings and acceptability among general physicians. Based on these results a scoring-based system can be developed in future studies which can indeed predict the development of GDM. This would prove invaluable in the early identification of this vulnerable group and in devising appropriate intervention strategies.

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None.

Authors' contribution

The concept of the study was given by JBK, AS, JSS and SM. JBK, AS, JSS, SM, MS, SR were involved in study design, critical discussion. AM, JS, SM and SG contributed to the study execution, data analysis. AM and JS were involved in statistics, representation of data and manuscript drafting. All authors were involved in the preparation of the manuscript, proof reading and approved the final manuscript.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

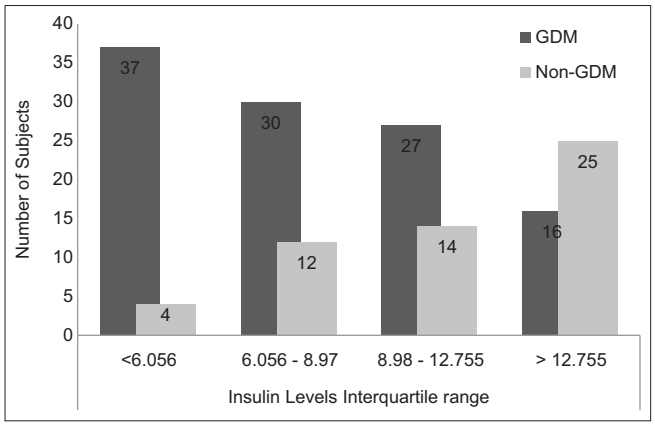
The datasets analysed during the study are available from the corresponding author upon reasonable request.

References

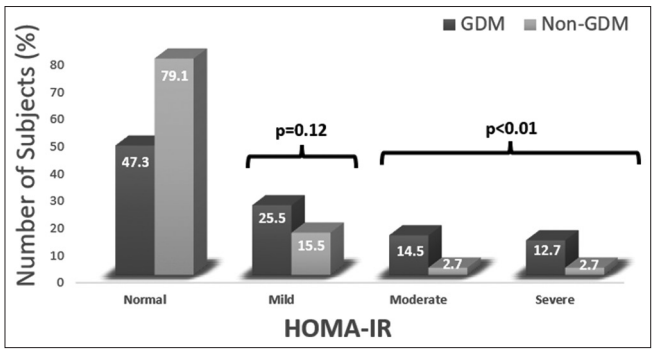
1. Metzger BE, Coustan DR, Organising Committee. Summary and recommendations of the Fourth International Workshop Conference on Gestational Diabetes. Diabetes Care 1998;21(Suppl 2):B161-7.
2. Dornhorst A, Paterson CM, Nicholls JSD, Wadsworth J, Chiu DC, Elkeles RS, *et al*. High prevalence of gestational diabetes in women from ethnic minority groups. Diabet Med 1992;9:820–5.
3. American Diabetes Association Professional Practice Committee. 6. Glycemic targets: Standards of medical care in diabetes—2022. Diabetes Care 2022;45(Suppl 1):S83–96.
4. Vounzoulaki E, Khunti K, Abner SC, Tan BK, Davies MJ, Gillies CL. Progression to type 2 diabetes in women with a known history of gestational diabetes: Systematic review and meta-analysis. BMJ 2020;369:m1361.
5. Pettitt DJ, Knowler WC. Long-term effects of the intrauterine environment, birth weight, and breast-feeding in Pima Indians. Diabetes Care 1998;21(Suppl 2):B138–41.
6. Cosson E, Benbara A, Pharisien I, Nguyen MT, Revaux A, Lormeau B, *et al*. Diagnostic and prognostic performances over 9 years of a selective screening strategy for gestational diabetes mellitus in a cohort of 18,775 subjects. Diabetes Care 2013;36:598–603.
7. van Hoorn F, Koster M, Naaktgeboren CA, Groenendaal F, Kwee A, Lamain-de Ruiter M, *et al*. Prognostic models versus single risk factor approach in first-trimester selective screening for gestational diabetes mellitus: A prospective population-based multicentre cohort study. BJOG 2021;128:645-54.
8. International Association of Diabetes and Pregnancy Study Groups consensus panel (IADPSG). International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycaemia in pregnancy. Diabetes Care 2010;33:676–82.
9. White SL, Lawlor DA, Briley AL, Godfrey KM, Nelson SM, Oteng-Ntim E, *et al*. Early antenatalprediction of gestational diabetes in obese women: Development of prediction tools for targeted intervention. PLoS One 2016;11:e0167846.
10. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
11. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, *et al*. Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402-10.
12. Yu X, Wang L, Zhang W, Ming J, Jia A, Xu S, *et al*. Fasting triglycerides, and glucose index is more suitable for the identification of metabolically unhealthy individuals in the Chinese adult population: A nationwide study. J Diabetes Investig 2019;10:1050–8.
13. Bogdanet D, Reddin C, Murphy D, Doheny HC, Halperin JA, Dunne F, *et al*. Emerging protein biomarkers for the diagnosis or

prediction of gestational diabetes—A scoping review. J Clin Med 2021;10:1533.
14. van Leeuwen M, Opmeer B, Zweers E, van Ballegooie E, ter Brugge H, de Valk H, *et al*. Estimating the risk of gestational diabetes mellitus: A clinical prediction model based on patient characteristics and medical history. BJOG 2010;117:69–75.
15. Thériault S, Forest JC, Massé J, Giguère Y. Validation of early risk-prediction models for gestational diabetes based on clinical characteristics. Diabetes Res Clin Pract 2014;103:419–25.
16. Madhavan A, Beena Kumari R, Sanal MG. A pilot study on the usefulness of body mass index and waist hip ratio as a predictive tool for gestational diabetes in Asian Indians. Gynecol Endocrinol 2008;24:701–7.
17. Takmaz T, Yalvaç ES, Özcan P, Çoban U, Karasu AFG, Ünsal M. The predictive value of weight gain and waist circumference for gestational diabetes mellitus. Turk J Obstet Gynecol 2019;16:199-204.
18. Song Z, Cheng Y, Li T, Fan Y, Zhang Q, Cheng H. Prediction of gestational diabetes mellitus by different obesity indices. BMC Pregnancy Childbirth 2022;22:288.
19. Brite J, Shiroma EJ, Bowers K, Yeung E, Laughon SK, Grewal JG, *et al*. Height and the risk of gestational diabetes: Variations by race/ethnicity. Diabet Med 2014;31:332-40.
20. Laughon SK, Catov J, Provins T, Roberts JM, Gandley RE. Elevated first-trimester uric acid concentrations are associated with the development of gestational diabetes. Am J Obstet Gynecol 2009;201:402-e1.
21. Ozgu-Erdinc AS, Yilmaz S, Yeral MI, Seckin KD, Erkaya S, Danisman AN. Prediction of gestational diabetes mellitus in the first trimester: Comparison of C-reactive protein, fasting plasma glucose, insulin and insulin sensitivity indices. J Matern Fetal Neonatal Med 2015;28:1957–62.
22. Lekva T, Godang K, Michelsen AE, Qvigstad E, Normann KR, Norwitz ER, *et al*. Prediction of gestational diabetes mellitus and pre-diabetes 5 years postpartum using 75 g oral glucose tolerance test at 14–16 weeks' gestation. Sci Rep 2018;8:13392.
23. Phaloprakarn C, Tangjitgamol S. Use of oral glucose tolerance test in early pregnancy to predict late-onset gestational diabetes mellitus in high-risk women. J Obstet Gynaecol Res 2008;34:331–6.
24. Ye M, Liu Y, Cao X, Yao F, Liu B, Li Y, *et al*. The utility of HbA1c for screening gestational diabetes mellitus and its relationship with adverse pregnancy outcomes. Diabetes Res Clin Pract 2016;114:43–9.
25. Correa PJ, Venegas P, Palmeiro Y, Albers D, Rice G, Roa J, *et al*. First trimester prediction of gestational diabetes mellitus using plasma biomarkers: A case-control study. J Perinat Med 2019;47:161–8.
26. Falcone V, Kotzaeridi G, Breil MH, Rosicky I, Stopp T, Yerlikaya-Schatten G, *et al*. Early assessment of the risk for gestational diabetes mellitus: Can fasting parameters of glucose metabolism contribute to risk prediction? Diabetes Metab J 2019;43:785-93.
27. Vasques ACJ, Novaes FS, de Oliveira M da S, Souza JRM, Yamanaka A, Pareja JC, *et al*. TyG index performs better than HOMA in a Brazilian population: A hyperglycemic clamp validated study. Diabetes Res Clin Pract 2011;93:e98–100.
28. Song T, Su G, Chi Y, Wu T, Xu Y, Chen C. Triglyceride–glucose index predicts the risk of gestational diabetes mellitus: A systematic review and meta-analysis. Gynecol Endocrinol 2022;38:10–5.
29. Hu J, Gillies CL, Lin S, Stewart ZA, Melford SE, Abrams KR, *et al*. Association of maternal lipid profile and gestational diabetes mellitus: A systematic review and meta-analysis of 292 studies and 97,880 women. EClinicalMedicine 2021;34:100830.
30. Nanda S, Savvidou M, Syngelaki A, Akolekar R, Nicolaides KH. Prediction of gestational diabetes mellitus by maternal factors and biomarkers at 11 to 13 weeks. Prenat Diagn 2011;31:135–41.

Supplementary Materials



Supplementary Figure 1: Distribution of Subjects depending on Insulin Interquartile range



Supplementary Figure 2: HOMA-IR Stratification among GDM and Non-GDM Controls