RESEARCH PAPER

Association of First-Trimester C-Peptide and HOMA-IR with Subsequent Gestational Diabetes Mellitus: A Prospective Observational Study

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ABSTRACT

Background:

Early prediction of Gestational Diabetes Mellitus (GDM) remains challenging, as routine screening is typically performed between 24–28 weeks of gestation. Identifying metabolic differences during early pregnancy may help improve understanding of the risk of subsequent GDM. This study investigated the association between first-trimester C-peptide levels and subsequent GDM.

Methods:

This prospective observational study with a nested case-control design was conducted at Sri Ramachandra Institute of Higher Education and Research between April 2024 and April 2026. 83 women who developed GDM and 83 age-matched normoglycemic controls were included in the study. First-trimester C-peptide, insulin, HOMA-IR and glycaemic parameters were assessed. Group comparisons, correlation analysis and multivariable logistic regression were performed.

Results:

First-trimester C-peptide concentrations were significantly higher in women who subsequently developed GDM than in controls with the median values [1.82 (1.24–2.77) vs. 1.39 (0.91–1.84) $p<0.001$] ng/mL. The GDM group also had significantly higher fasting insulin, HOMA-IR, fasting blood glucose, postprandial blood glucose and HbA1c levels ($p<0.001$). C-peptide was positively correlated with fasting insulin ($\rho=0.625$, $p<0.001$), HOMA-IR ($\rho = 0.633$, $p<0.001$), BMI ($\rho = 0.212$, $p=0.009$), fasting blood glucose ($\rho = 0.178$, $p=0.025$) and postprandial blood glucose ($\rho = 0.227$, $p=0.005$). After adjustment for maternal age, BMI, family history of diabetes mellitus and previous history of GDM, C-peptide remained significantly associated with GDM (adjusted OR: 2.545, 95% CI: 1.607–4.029; $p<0.001$).

Conclusion:

First-trimester C-peptide levels were higher in women who subsequently developed GDM and remained associated with GDM after adjustment for maternal age and BMI. Further prospective studies are needed to evaluate its predictive performance and potential clinical utility.

Keywords: C-peptide; Gestational diabetes mellitus; Insulin resistance; Prediction; Biomarkers; Pregnancy; Healthcare (SDG 3)
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INTRODUCTION

Gestational diabetes mellitus (GDM) is a metabolic disorder characterised by glucose intolerance first recognised during pregnancy (1). Its pathophysiology involves increased insulin resistance through placental hormones and insufficient insulin secretion, as pregnancy progresses, physiological changes in insulin sensitivity increase the demand for insulin secretion. When pancreatic beta-cell compensation is insufficient to maintain glucose homeostasis, hyperglycaemia may develop (2). The global prevalence of GDM is steadily increasing, with approximately 14-15% of pregnancies documented to be affected, which accounts for nearly 20 million births worldwide. The International Diabetes Federation (IDF) 2024 report cites a global prevalence of GDM to be 15.6%, with 24.9% in South-East Asia and 26.1% in India, which suggests considerable regional variation (3). The increasing burden can largely be attributed to sedentary lifestyles, obesity, advanced maternal age and genetic predisposition, contributing to β-cell dysfunction and dysregulated glucose (1,4).

GDM is associated with maternal outcomes, including gestational hypertension, pre-eclampsia, and caesarean delivery, as well as fetal outcomes including macrosomia and the potential for obesity and type 2 diabetes mellitus later in life. Although GDM is typically resolved by the postpartum period, women with a history of GDM remain at high risk for metabolic syndrome and developing type 2 diabetes, which highlights the clinical and public health significance of this condition (2,5). Although there is considerable research, the mechanisms that cause GDM are still complex due to complex hormones, metabolism, and inflammatory interactions. Early identification of metabolic changes, particularly during the first trimester, may help improve understanding of the processes associated with subsequent GDM and support the development of early risk-assessment strategies (1,4).

C-peptide (Connecting Peptide), a 31-amino-acid peptide released during the conversion of proinsulin to insulin and is secreted into the circulation alongside endogenous insulin. It is released along with insulin and remains in the blood longer, making it a useful indicator of insulin secretion by pancreatic beta cells (6,7). Its concentrations may increase when pancreatic beta cells compensate for reduced insulin sensitivity. Consequently, elevated early-pregnancy C-peptide may reflect underlying metabolic changes associated with the subsequent development of GDM, although the concentration alone does not distinguish compensatory insulin secretion from beta-cell dysfunction (8,9).

Evidence supporting the relationship between C-peptide and GDM has emerged from prospective studies found that higher maternal C-peptide levels were associated with an increased risk of subsequent GDM (10–12). These findings suggest that C-peptide may reflect early maternal metabolic changes before routine GDM screening. However, further studies in different populations are needed to establish its clinical relevance. Assessing its relationship with insulin, insulin resistance and glycaemic parameters may help clarify its role in GDM. Therefore, the present study aimed to compare first-trimester C-peptide concentrations between pregnant women who subsequently developed GDM and age-matched normoglycemic pregnant women, examine correlations between C-peptide and clinical and biochemical parameters, and evaluate its association with subsequent GDM after adjustment for potential confounders.

MATERIALS AND METHODS

Study Design and setting: This Prospective observational study with Nested Case-control design was carried out at the Department of Biochemistry and Department of Obstetrics and Gynaecology at Sri Ramachandra Institute of Higher Education and Research between April 2024 and April 2026. Ethical approval was obtained from the Institutional Ethics Committee prior to the commencement of the study (IEC-NI/23/NOV/90/119). Written informed

consent was obtained from all participants. Participant confidentiality was strictly maintained.

Inclusion and Exclusion Criteria: Pregnant women aged 18–40 years with a singleton pregnancy, gestational age <13 weeks at recruitment, visiting the Obstetrics and Gynaecology outpatient department for routine antenatal care during the first trimester were included in the study.

Pregnant women with pre-existing diabetes mellitus, metabolic syndrome, hypertension, cardiovascular disease, thyroid disorders diagnosed prior to pregnancy, hematological disorders or polycystic ovary syndrome and Women with active infections, chronic inflammatory diseases, cancer, tuberculosis, HIV, or other major infectious diseases also women with Multiple pregnancies and pregnancies conceived through assisted reproductive techniques such as in vitro fertilization (IVF) or intrauterine insemination (IUI) were excluded.

Study Participants, Recruitment and Follow-up: All the pregnant women attending the Obstetrics and Gynaecology outpatient department for routine antenatal care during the first trimester were screened based on the predefined inclusion and exclusion criteria and eligible participants were recruited in this study.

Blood samples were collected during routine first-trimester investigations and stored until analysis. All the recruited participants were prospectively followed up to 24-28 weeks of gestation. When a 75-g Oral Glucose Tolerance Test (OGTT) was performed for the diagnosis of GDM. GDM was diagnosed according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria. Participants meeting one or more of the following plasma glucose values were diagnosed with GDM (Fasting plasma glucose ≥ 92 mg/dL or 1-hour plasma glucose ≥ 180 mg/dL or 2-hour plasma glucose ≥ 153 mg/dL).

A total of 497 pregnant women were recruited in this study. Among them, 381 participants completed their follow-up and underwent OGTT. Based on the IADPSG criteria, 83 women were diagnosed with GDM, while 298 women did not have GDM.

Case and Control Selection: A nested case-control analysis was performed within the prospectively followed cohort. All 83 women diagnosed with GDM were included as Cases. A total of 83 age-matched normoglycemic women were selected from the 298 women who did not develop GDM, using an age-matching range of ±2 years. The final analysis, included 166 participants: 83 women in the GDM group and 83 age-matched normoglycaemic women in the non-GDM group. **Figure 1** presents the study flowchart, illustrating participant recruitment, follow-up, GDM diagnosis, and selection of the final study population.

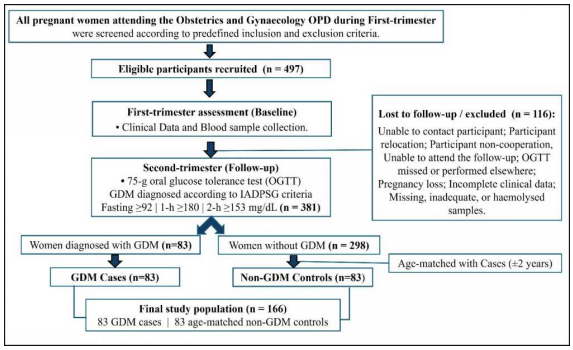


Figure 1. Flowchart of participant recruitment, follow-up, and selection of the final study population. Abbreviations: OPD, outpatient department; OGTT, oral glucose tolerance test; IADPSG, International Association of Diabetes and Pregnancy Study Groups.

Data Collection: The baseline demographic, clinical characteristics and obstetric history were collected from participants and also from their medical records. The variables such as age, gestational age, BMI, family history of diabetes mellitus, Gravidity, Previous history of GDM, fasting blood glucose, postprandial blood glucose and HbA1c levels were recorded.

Sample collection and processing: The blood samples were collected from study participants during first assessments and stored at -80°C until further analysis. Fasting blood glucose, postprandial blood glucose, HbA1c, fasting insulin and C-peptide were assessed using measurements obtained during the first-trimester samples, before GDM status was determined by the 75-g OGTT at 24–28 weeks of gestation. Glucose parameters were measured using the enzymatic hexokinase method on an automated clinical chemistry analyzer. C-peptide and insulin were measured using the electrochemiluminescence immunoassay (ECLIA) method on a cobas e automated immunoassay analyzer. The measurement range was 0.010 - 40 ng/mL for C-Peptide and 0.2 - 1000 µIU/mL for insulin. Sample handling and storage conditions were maintained throughout the study to ensure the sample integrity.

The fasting insulin and fasting blood glucose measurements were used to calculate the homeostatic model assessment of insulin resistance (HOMA-IR) using the following formula: HOMA-

IR = [Fasting insulin (μIU/mL) × Fasting glucose (mg/dL)] / 405

Sample Size: The recruitment sample size was calculated based on the expected prevalence of GDM using the single-proportion formula in NMaster 2.0, assuming 22.8% GDM prevalence (13), 95% confidence interval and 4% precision. After adjusting for anticipated 15% loss to follow-up, the target recruitment sample size was estimated to be 497 pregnant women for this study.

Statistical analysis: Using IBM SPSS Statistics version 21.0 the analysis was performed. All the parameters were assessed for distribution of data using the Shapiro–Wilk test, normally distributed data presented as mean and standard deviation (SD) while non-normally distributed presented as median with interquartile range (IQR) then categorical variables was presented as frequencies and percentages. Comparisons between two groups were performed using the independent samples t-test or Mann–Whitney U test or Chi-square test based on variables and data distribution. To evaluate the association between first-trimester C-Peptide and GDM, logistic regression analysis was performed and results were expressed as odds ratios (OR) with 95% confidence intervals (CI). A p-value < 0.05 was considered statistically significant.

RESULTS

Baseline Characteristics of Study Participants

A total of 166 participants were included in this study, 83 women in the GDM group and 83 age-matched normoglycaemic women in the non-GDM group. The baseline clinical and obstetric characteristics of the study participants are presented in Table 1. Maternal age and gestational age at recruitment were comparable between the GDM and non-GDM groups (p = 0.581 and p = 0.896, respectively). However, the mean BMI was significantly higher in the GDM group than in the non-GDM group (26.3 ± 3.57 vs. 24.6 ± 2.90 kg/m², p = 0.001). A family history of diabetes mellitus was more frequent among women who developed GDM than among controls. No, statistically significant differences were observed in gravidity or previous history of GDM among multigravida women.

Table 1. Clinical and Obstetric Characteristics of the Study Participants

Characteristics		GDM Cases (n=83)	Non-GDM Controls (n=83)	p-value
Maternal Age (years)		28.3 ± 4.44	27.9 ± 4.26	0.581
BMI (kg/m²)		26.3 ± 3.57	24.6 ± 2.90	0.001**
Gestational Age (weeks)		8.9 ± 1.67	8.9 ± 1.78	0.896
Family History of Diabetes Mellitus [n (%)]	No	54 (65.1 %)	74 (89.2 %)	<0.001 ***
	Yes	29 (34.9 %)	9 (10.8 %)	
Gravidity [n (%)]	Primigravida	28 (33.7 %)	39 (47.0 %)	0.082
	Multigravida	55 (66.3 %)	44 (53.0 %)	
Previous History of GDM [n (%)]	No	39 (70.9 %)	37 (84.1 %)	0.123
	Yes	16 (29.1 %)	7 (15.9 %)	

Note: Continuous variables are presented as mean ± standard deviation (SD), while categorical variables are presented as frequency [n (%)]. Independent samples t-test was used for comparison of continuous variables, and Chi-square test was used for comparison of categorical variables between the GDM and non-GDM groups; Significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

First-Trimester Biochemical Characteristics

The biochemical characteristics of the study participants are presented in Table 2 and Figure 2. Fasting blood glucose (FBG), postprandial blood glucose (PPBG), and glycated haemoglobin (HbA1c) levels were significantly higher in the GDM group than in the non-GDM group (p < 0.001). Fasting insulin concentrations were significantly higher in the GDM group than in the non-GDM group, with median values of [16.59 (11.00–29.72) vs. 9.73 (6.73–14.59) μIU/mL, p < 0.001]. Similarly, HOMA-IR was significantly higher in the GDM group than in the non-GDM group [3.75 (2.37–6.66) vs. 1.98 (1.36–2.99), p <

0.001]. First-trimester C-peptide concentrations were also significantly higher among participants in the GDM group than in the non-GDM group [1.82 (1.24–2.77] vs. 1.39 (0.91–1.84) ng/mL, p < 0.001].

Table 2. Biochemical Characteristics of the Study Participants

Parameters (Unit)	GDM Group (n=83)	Non-GDM Group (n=83)	p-value
FBG (mg/dL)	88.7 ± 9.32	83.2 ± 6.03	<0.001***
PPBG (mg/dL)	123.3 ± 18.17	105.5 ± 19.47	<0.001***
HBA1C (%)	5.45 ± 0.26	5.14 ± 0.32	<0.001***
Fasting Insulin (µIU/mL)	16.59 (11.00–29.72)	9.73 (6.73–14.59)	<0.001***
HOMA-IR	3.75 (2.37–6.66)	1.98 (1.36–2.99)	<0.001***
C-Peptide (ng/mL)	1.82 (1.24–2.77)	1.39 (0.91–1.84)	<0.001***

Note: Values are presented as mean ± standard deviation (SD) for normally distributed variables and median (interquartile range [IQR]) for non-normally distributed variables. **Abbreviations:** FBG, fasting blood glucose; PPBG, postprandial blood glucose; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; C-peptide, connecting peptide; ng/mL, nanograms per millilitre; mg/dL, milligrams per decilitre; µIU/mL, microunits per millilitre.

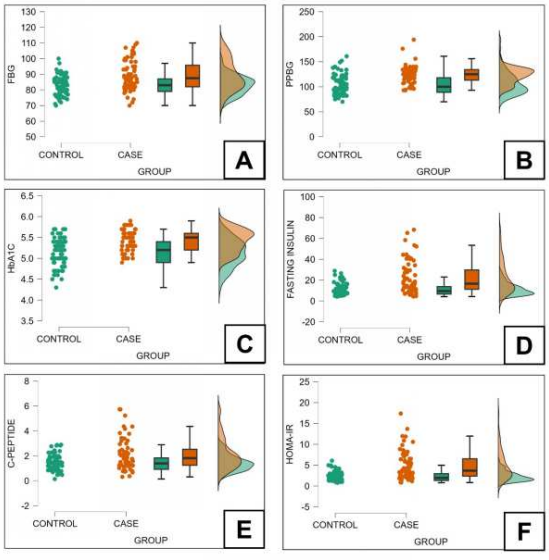


Figure 2. Comparison of first-trimester biochemical parameters between the GDM and non-GDM groups. The figure compares the distributions of biochemical parameters between the GDM (case) and non-GDM (control) groups: **(A)** fasting blood glucose (FBG), **(B)** postprandial blood glucose (PPBG), **(C)** glycated haemoglobin (HbA1c), **(D)** fasting insulin, **(E)** C-peptide, and **(F)** homeostatic model assessment of insulin resistance (HOMA-IR). Individual data points, box plots, and distribution plots are shown for each parameter in both groups. The figure illustrates the differences in the distribution and variability of the biochemical parameters between the groups.

Correlation Between C-Peptide and Clinical and Biochemical Parameters

The correlations between C-peptide and clinical and biochemical parameters in the study population are presented in Table 3 and Figure 3. C-peptide showed a moderate positive correlation with fasting insulin (ρ = 0.625, p < 0.001) and HOMA-IR (ρ = 0.633, p < 0.001). Weak positive correlations were observed between C-peptide and BMI (ρ = 0.212, p = 0.009), FBG (ρ = 0.178, p = 0.025), and PPBG (ρ = 0.227, p = 0.005). However, the correlations between C-peptide and maternal age (ρ = 0.124, p = 0.120) and HbA1c (ρ = 0.145, p = 0.076) were not statistically significant. Figure 3 further illustrates the positive relationships between C-peptide and fasting insulin and between C-peptide and HOMA-IR using scatterplots.

Table 3. Correlation of C-peptide with clinical and biochemical parameters in the study population.

Parameter	C-Peptide	
	Spearman's rho (r)	p-value
Age	0.124	0.12
BMI	0.212	0.009**
FBG	0.178	0.025*
PPBG	0.227	0.005**
HbA1C	0.145	0.076
Fasting Insulin	0.625	<0.001***
HOMA-IR	0.633	<0.001***

Note: Correlation coefficients were calculated using Spearman's rank correlation. $p < 0.05$ was considered statistically significant. FBG, fasting blood glucose; PPBG, postprandial blood glucose;

HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance.

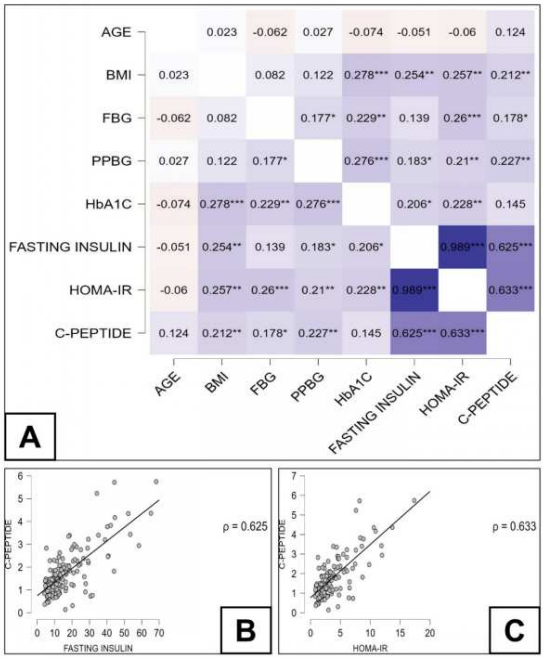


Figure 3. Correlation of C-peptide with clinical and biochemical parameters in the study population. **(A)** Heatmap showing Spearman's rank correlation coefficients between maternal age, BMI, FBG, PPBG, HbA1c, fasting insulin, HOMA-IR, and C-peptide. **(B)** Scatterplot showing the positive correlation between C-peptide and fasting insulin. **(C)** Scatterplot showing the positive correlation between C-peptide and HOMA-IR. Abbreviations: BMI, body mass index; FBG, fasting blood glucose; PPBG, postprandial blood glucose; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance.

Logistic Regression Analysis

Logistic regression was performed to examine the association of first-trimester C-peptide with GDM (Table 4). In univariate logistic regression analysis, BMI, family history of diabetes mellitus, previous history of GDM and C-peptide (OR: 2.295, 95% CI: 1.518–3.470; $p < 0.001$) were significantly associated with GDM. In multivariable logistic regression analysis, C-peptide remained significantly associated with GDM after adjustment for maternal age, BMI, family history of diabetes mellitus and previous history of GDM (adjusted OR: 2.545, 95% CI: 1.607–4.029; $p < 0.001$). Family history of diabetes mellitus also remained significantly associated with GDM ($p < 0.001$). BMI ($p = 0.059$), maternal age ($p = 0.791$) and previous history of GDM ($p = 0.937$) were not statistically significant in the adjusted model. Maternal age was not significantly associated with GDM, this finding may partly reflect the individual age-matching of cases and controls, which reduced age differences between the groups.

Table 4. Binary logistic regression analysis of first-trimester C-peptide and GDM.

Univariate Analysis		
Variable	**Unadjusted OR (95% CI)**	**p-value**
Age	1.020 (0.951-1.095)	0.578
BMI	1.179 (1.061-1.309)	0.002
Family History of DM	4.416 (1.933-10.086)	<0.001
Previous History of GDM	2.593 (1.006-6.684)	0.049
C-Peptide	2.295 (1.518-3.470)	**<0.001*** **
Multivariate Analysis		
Variable	**Adjusted OR (95% CI)**	**p-value**
Age	1.011 (0.931-1.098)	0.791
BMI	1.118 (0.996-1.256)	0.059
Family History of DM	6.367 (2.412-16.807)	<0.001
Previous History of GDM	0.954 (0.295-3.082)	0.937
C-Peptide	2.545 (1.607-4.029)	**<0.001*** **

Note: Data are presented as odds ratio (OR) with 95% confidence interval (CI). Crude ORs were obtained from univariate logistic regression analysis. Adjusted ORs were obtained from multivariable logistic regression analysis using the Enter method. Abbreviations: OR, Odds Ratio; CI, Confidence Interval; BMI, Body Mass Index.

DISCUSSION

The present study examined the relationship between first-trimester C-peptide concentrations and subsequent GDM. We found C-peptide concentrations were significantly higher in women who later developed GDM than in normoglycemic controls. These women also had higher fasting insulin, HOMA-IR, fasting blood glucose,

postprandial blood glucose and HbA1c levels. Notably, the association between C-peptide and GDM remained significant after adjustment for confounding factors such as maternal age and BMI. These findings suggest that changes in insulin secretion and metabolic status may already be present during early pregnancy among women who subsequently develop GDM.

The findings of this study are consistent with previous findings reported by Yang et al., (2022) who investigated early-pregnancy serum C-peptide in a nested case–control study involving 332 women with GDM and 664 controls. After adjustment for potential confounders, women in the highest C-peptide quartile had 2.28 times the odds of GDM compared with those in the lowest quartile (95% CI: 1.43–3.62; p<0.001). They also found that adding C-peptide to a model containing conventional risk factors modestly improved the C-statistic from 0.63 to 0.66 (p = 0.008) (10). Similarly, Zhao et al., (2024) studied fasting serum C-peptide in 218 women with GDM and 218 controls. They reported that a one-unit increase in log-transformed C-peptide was associated with higher odds of GDM during both the first trimester (OR: 2.38, 95% CI: 1.33–4.40) and the second trimester (OR: 3.07, 95% CI: 1.49–6.62). The area under the ROC curve was 0.804 for first-trimester C-peptide and 0.824 for second-trimester C-peptide (11).

In the present study, median first-trimester C-peptide concentrations were 1.82 (1.24–2.77) ng/mL in the GDM group and 1.39 (0.91–1.84) ng/mL in the non-GDM group (p < 0.001). The higher concentrations observed among women who subsequently developed GDM are consistent with the findings of both previous studies. Although the magnitude of the associations cannot be directly compared because of differences in study populations and statistical methods, the findings provide further evidence of a relationship between early-pregnancy C-peptide and subsequent GDM.

C-peptide showed weak positive correlations with BMI (ρ = 0.212, p = 0.009), fasting blood glucose (ρ = 0.178, p = 0.025) and postprandial blood glucose (ρ = 0.227, p = 0.005). Stronger correlations were observed with fasting insulin (ρ = 0.625, p < 0.001) and HOMA-IR (ρ = 0.633, p < 0.001). The correlation with fasting insulin is expected because fasting insulin, which is physiologically related to C-peptide secretion, is also a component of the HOMA-IR calculation. No significant correlations were observed between C-peptide and HbA1c (ρ = 0.145, p = 0.076) or maternal age (ρ = 0.124, p = 0.120). Overall, these findings, suggests that C-peptide was more closely related to fasting insulin and HOMA-IR than with the other glycaemic and clinical parameters assessed in this study.

In the logistic regression analysis, first-trimester C-peptide was significantly associated with subsequent GDM, and this association remained significant after adjustment for confounding factors such as maternal age, BMI, family history of diabetes mellitus and previous history of GDM. This finding suggests that C-peptide may reflect early metabolic changes associated with the development of GDM.

C-peptide is released alongside insulin during the processing of proinsulin. Beyond its established role as a marker of insulin secretion, C-peptide has been investigated for potential biological effects on cellular signalling and gene expression (6,7). Experimental studies suggest that it may interact with specific cell-surface receptors and influence intracellular pathways involved in cellular function (8,14). However, the clinical relevance of these effects remains to be fully established. In the present study, higher C-peptide concentrations were associated with subsequent GDM and positively correlated with fasting insulin and HOMA-IR. These findings may reflect early pregnancy metabolic differences in insulin secretory activity and insulin resistance during early pregnancy. However, as the study did not investigate the direct biological actions of C-peptide, these findings do not establish that c-peptide itself causes insulin resistance or contributes directly to the development of GDM. Overall, the results support further investigation of first-trimester C-peptide as indicator for identifying women at increased risk of GDM. However, its predictive performance and clinical utility require further evaluation in larger, independent cohorts.

Clinical implications:

Measuring C-peptide during the first trimester may help identify metabolic differences before routine GDM screening at 24–28 weeks. However, this study shows an association between C-peptide and GDM and does not establish its usefulness as a screening marker. A C-peptide cut-off, sensitivity, specificity and improvement in prediction beyond established clinical factors were not evaluated. Further studies involving larger prospective cohorts are needed to assess its clinical value.

Future studies can evaluate whether C-peptide improves GDM prediction when combined with established clinical and biochemical factors. They should also identify suitable cut-off values, validate the findings in independent populations and assess whether repeated measurements during pregnancy provide additional information.

Strengths and limitations:

The prospective design and follow-up until GDM diagnosis at 24–28 weeks are key strengths of this study. Group comparisons, correlation analysis and multivariable regression provided a comprehensive assessment of the association between C-peptide and maternal metabolic status.

Limitations include the single-centre design and relatively small sample size, which may limit generalisability. The equal numbers of GDM cases and controls do not reflect GDM prevalence in the original cohort. C-peptide levels were measured only in the first trimester and its diagnosis performance and Predictive values were not evaluated or validated in an independent cohort.

CONCLUSIONS

In this prospective observational study with a nested case-control analysis, first-trimester C-peptide concentrations were significantly higher among women who subsequently developed GDM than normoglycemic controls. C-peptide was positively correlated with fasting insulin, HOMA-IR, BMI and glucose parameters, and remained significantly associated with GDM even after adjustment for potential confounders. These findings support further investigation of C-peptide as a potential indicator of early-pregnancy metabolic status associated with subsequent GDM. Larger multicentre prospective studies, including independent validation and assessment of predictive performance, are required before its clinical use can be recommended.

Conflict of interest: The authors declare no potential conflict of interest.

Ethical Approval: The study was approved by the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (IEC-NI/23/NOV/90/119) before commencement of the study and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrolment. Participant confidentiality was maintained throughout the study, and identifying information was not disclosed.

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