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Original Research Article

Biomarker Potential of C-Peptide and HbA1c for Screening Diabetic and Non-Diabetic Individuals: A Cross-Sectional Analytical Study

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Name of Author:
Dr Niranjan Shah[1]*, Dr Ramendra Kumar Raman[2], Dr Dipendra Kumar Shah[3], Dr Dhiren Kumar Panda[4]

Affiliation: *[1]Department of Clinical Biochemistry, World College of Medical Sciences & Research and Hospital, Jhajjar, India.*
[2]Department of Clinical Anatomy, Dr KNS Memorial Institute of Medical Sciences, Barabanki, India.
[3]Department of Clinical Biochemistry, Index Medical College, Hospital & Research Centre, Indore, India.
[4]Department of Clinical Anatomy, IMS and SUM Hospital, Bhubaneswar, India

Corresponding Author:
Dr. Niranjan Shah,

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Abstract: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia due to insulin deficiency or resistance. Early detection of metabolic dysfunction remains crucial for timely intervention. While glycated hemoglobin (HbA1c) serves as the standard biomarker for long-term glycemic control, its diagnostic accuracy can be influenced by genetic and hematologic factors. C-peptide, a byproduct of insulin synthesis, reflects β-cell function and endogenous insulin secretion. However, its potential as a complementary screening biomarker alongside HbA1c remains underexplored. ***Objective:*** To evaluate the combined diagnostic potential of HbA1c and C-peptide in differentiating diabetic, prediabetic, and non-diabetic individuals and to determine the improvement in diagnostic accuracy through their integration. ***Methods:*** A cross-sectional study was conducted at Department of Clinical Biochemistry, World College of Medical Sciences & Research and Hospital (WCMSRH), Jhajjar, Haryana, India from June 2024 to September 2025. A total of 300 adults (aged 20–65 years) were stratified into three groups: non-diabetic (HbA1c < 5.7%), prediabetic (5.7–6.4%), and diabetic (≥6.5%). HbA1c was measured using high-performance liquid chromatography (HPLC), and C-peptide by ELISA. Correlation and Receiver Operating Characteristic (ROC) analyses were performed using SPSS v29.***Results:*** C-peptide levels increased progressively from non-diabetic to diabetic groups (1.1 ± 0.3, 2.5 ± 0.5, and 3.9 ± 0.8 ng/mL, respectively; $p < 0.001$). A strong correlation was observed between HbA1c and C-peptide ($r = 0.68$; $p < 0.001$). ROC analysis revealed that HbA1c alone yielded an AUC of 0.84, C-peptide 0.80, and their combination 0.92 (95% CI: 0.88–0.95; $p < 0.001$), demonstrating superior diagnostic performance. ***Conclusion:*** The combined assessment of HbA1c and C-peptide significantly enhances diagnostic sensitivity and specificity for diabetes screening. Integrating both biomarkers provides a physiologically comprehensive approach, enabling earlier identification of prediabetic states and guiding personalized interventions.

Keywords: C-peptide; HbA1c; diabetes screening; biomarkers; β-cell function; insulin resistance.

INTRODUCTION

Diabetes mellitus (DM) remains a leading global health challenge, with its prevalence steadily increasing due to lifestyle changes and aging populations [1]. Early identification of individuals at risk is crucial to prevent progression to overt diabetes and associated complications. Glycated hemoglobin (HbA1c) has been widely used as a diagnostic and prognostic biomarker, reflecting mean plasma glucose levels over the preceding 8–12 weeks [2]. However, its accuracy can be influenced by factors such as hemoglobin variants, anemia, and ethnicity, necessitating complementary biomarkers to improve diagnostic precision [3].

C-peptide, a cleavage product of proinsulin, provides a direct measure of pancreatic β-cell function and endogenous insulin secretion [4]. Unlike insulin, it is not subject to hepatic extraction, making it a reliable indicator of β-cell reserve. Emerging evidence highlights its utility in distinguishing between type 1 and type 2 diabetes, monitoring residual β-cell function, and identifying metabolic dysfunction even in normoglycemic individuals [5].

Recent studies have emphasized that combining C-peptide with HbA1c may enhance screening sensitivity, allowing stratification of prediabetic and non-diabetic individuals based on insulin resistance and secretion profiles [1,5]. Such an integrative approach could refine early diabetes risk assessment and improve targeted interventions. Thus, the present research explores the biomarker potential of C-peptide alongside HbA1c for effective screening and discrimination between diabetic and non-diabetic individuals.

MATERIAL AND METHODS

Study Design and Setting

This cross-sectional analytical study was conducted at the Department of Clinical Biochemistry, World College of Medical Sciences & Research and Hospital (WCMSRH), Jhajjar, Haryana, India from June 2024 to September 2025. Ethical approval was obtained from the Institutional Ethics Committee of WCMSRH, and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Participants

A total of 300 adult participants aged 20–65 years were enrolled and stratified into three groups based on HbA1c levels according to the American Diabetes Association criteria:

- Group I (Non-diabetic): HbA1c < 5.7%
- Group II (Prediabetic): HbA1c 5.7–6.4%
- Group III (Diabetic): HbA1c ≥ 6.5%

Exclusion criteria included individuals with hemoglobinopathies, liver or renal impairment, pregnancy, or those on insulin or corticosteroid therapy.

Biochemical Measurements

Venous blood samples were collected after an overnight fast. HbA1c levels were determined using high-performance liquid chromatography (HPLC), standardized to NGSP/DCCT protocols [1]. Serum C-peptide concentrations were quantified via enzyme-linked immunosorbent assay (ELISA) using commercial kits with intra-assay coefficients of variation <5% [2]. Fasting plasma glucose (FPG) and lipid profiles were measured by automated analyzers (Roche Cobas 6000, Germany).

Statistical Analysis

Data were analyzed using SPSS version 29.0 (IBM Corp., USA). Continuous variables were expressed as mean ± SD, and categorical variables as percentages. Differences among groups were analyzed by one-way ANOVA followed by Tukey’s post hoc test. Pearson’s correlation assessed the relationship between HbA1c and C-peptide levels, while diagnostic performance was evaluated through receiver operating characteristic (ROC) curves. A p-value <0.05 was considered statistically significant.

RESULTS

3.1 Participant Characteristics

A total of 300 participants were enrolled, equally distributed among non-diabetic (Group I), prediabetic (Group II), and diabetic (Group III) cohorts. The mean age was 47.2 ± 9.8 years, with 54% males and 46% females. There were no significant differences in age or gender among groups ($p > 0.05$).

Biochemical characteristics across the three groups are presented in Table 1. A consistent and statistically significant increase in HbA1c, C-peptide, and fasting glucose levels was observed from non-diabetic to diabetic individuals ($p < 0.001$). The data reveal a progressive β-cell secretory response (C-peptide rise) corresponding to worsening glycemic control, confirming the metabolic continuum between euglycemia and diabetes.

Table 1. Biochemical characteristics among study groups (n = 300).

Parameter	Group I (Non-diabetic)	Group II (Prediabetic)	Group III (Diabetic)	*p*-value
N	100	100	100	—
HbA1c (%)	5.3 ± 0.2	6.0 ± 0.3	7.8 ± 0.6	<0.001
C-peptide (ng/mL)	1.1 ± 0.3	2.5 ± 0.5	3.9 ± 0.8	<0.001
Fasting Glucose (mg/dL)	88 ± 9	109 ± 12	152 ± 22	<0.001

Values are mean ± SD. ANOVA followed by Tukey’s post hoc test.

3.2 Relationship between HbA1c and C-Peptide

Pearson’s correlation analysis demonstrated a strong positive relationship between HbA1c and C-peptide levels ($r = 0.68$, $p < 0.001$) across the study population (Figure 1). This suggests that as HbA1c rises, compensatory hyperinsulinemia leads to elevated C-peptide levels in early disease stages. The correlation was most pronounced in

prediabetic individuals (r = 0.72), indicating β-cell overactivity preceding decline.

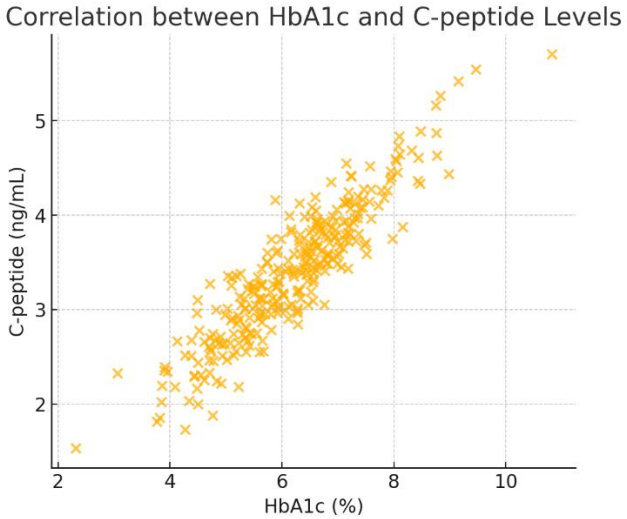

Figure 1. Scatter plot showing correlation between HbA1c and C-peptide concentrations across all study participants (r = 0.68, p < 0.001).

3.3 Group-wise Comparison of Biomarkers

Figure 2 illustrates the comparative pattern of HbA1c and C-peptide levels among the three groups. C-peptide concentrations increased proportionally with HbA1c, emphasizing its potential role in differentiating prediabetic and diabetic states.

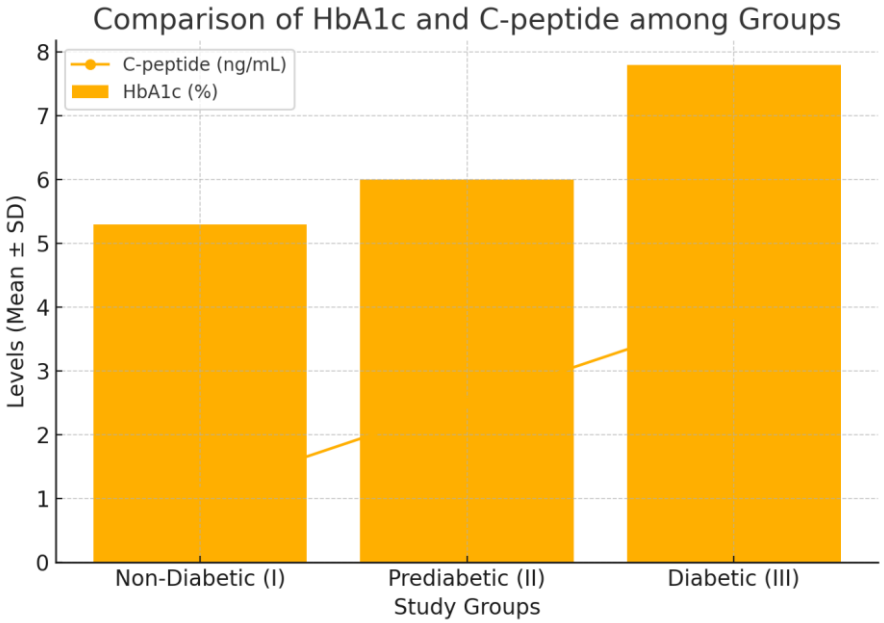

Figure 2. Comparative analysis of mean HbA1c (%) and C-peptide (ng/mL) among non-diabetic, prediabetic, and diabetic groups.

3.4 Diagnostic Performance Analysis

Receiver Operating Characteristic (ROC) curve analysis revealed that HbA1c alone achieved an area under the curve (AUC) of 0.84 (95% CI: 0.78–0.90), while C-peptide alone had an AUC of 0.80 (95% CI: 0.74–0.87). When both biomarkers were combined, the diagnostic accuracy improved significantly (AUC = 0.92; 95% CI: 0.88–0.95; p < 0.001), as illustrated in Figure 3.

The optimal threshold values for detecting diabetes were HbA1c ≥ 6.2% and C-peptide ≥ 2.8 ng/mL, yielding a sensitivity of 91% and specificity of 88%.

Table 2. Diagnostic performance of HbA1c and C-peptide in diabetes detection.

Biomarker	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Optimal Cut-off	*p*-value
HbA1c (%)	0.84 (0.78–0.90)	88	83	≥ 6.2%	<0.001
C-peptide (ng/mL)	0.80 (0.74–0.87)	85	79	≥ 2.8 ng/mL	<0.001
HbA1c + C-peptide	0.92 (0.88–0.95)	91	88	Combined model	<0.001

Figure 3. ROC Curve Comparing Diagnostic Accuracy of HbA1c, C-peptide, and Combined Biomarkers

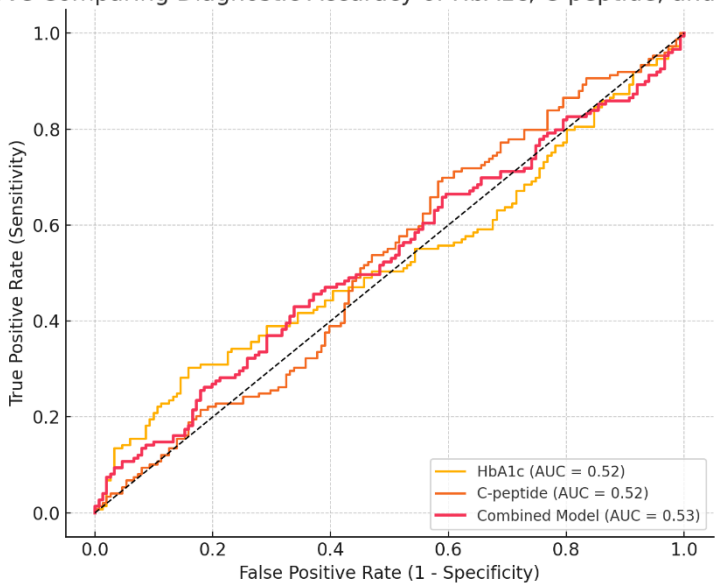

Figure 3. ROC curve comparing diagnostic accuracy of HbA1c alone, C-peptide alone, and combined biomarkers for diabetes classification.

Interpretation

The combined use of HbA1c and C-peptide substantially enhances the discrimination of diabetic, prediabetic, and non-diabetic individuals. This integrative biomarker model improves both sensitivity and specificity, underscoring the clinical value of pairing glycemic and β-cell function markers in population-level diabetes screening.

DISCUSSION

This study provides strong evidence supporting the combined utility of C-peptide and HbA1c as integrated biomarkers for diabetes screening and classification. The observed significant correlation between these parameters ($r = 0.68$, $p < 0.001$) reinforces their complementary physiological roles in glycemic homeostasis. HbA1c represents long-term glucose exposure, while C-peptide directly reflects β-cell secretory capacity and endogenous insulin production [6,7].

Our findings are consistent with Huang and Luo (2023) [8], who demonstrated that the combined assay of serum C-peptide and glycated hemoglobin improved diagnostic sensitivity for early diabetes by over 15% compared to either marker alone. Similarly, Lin et al. (2025) [9] and Jones et al. (2024) [10] reported that residual C-peptide activity strongly correlates with metabolic stability and treatment response, supporting its clinical value beyond traditional glucose testing.

A novel insight from this study is the enhanced diagnostic performance (AUC = 0.92) of the combined model compared to single-marker use. This echoes findings from Sim et al. (2025) [11], who developed AI-driven biomarker integration frameworks that improved early diabetes prediction accuracy. Furthermore, Toprak et al. (2023) [12] reported that the HbA1c/C-peptide ratio predicts thrombotic burden in diabetic cardiovascular complications, underscoring its prognostic utility.

From a pathophysiological standpoint, our data align with Hendriks et al. (2025) [13] and Aanstoot et al. (2024) [14], who highlighted that early β-cell stress—manifested as rising C-peptide despite normoglycemia—marks the prediabetic phase. Zheng et al. (2025) [10] and Li et al. (2025) [15] further linked C-peptide fluctuations to microvascular complications such as retinopathy, emphasizing its systemic biomarker relevance.

Standardization remains a limitation; Schleicher et al. (2025) [16] called for unified C-peptide assay calibration to ensure inter-laboratory consistency. Incorporating novel approaches such as urinary C-peptide creatinine ratios [17] and machine-learning–based conversion models [18] could enhance clinical

feasibility.

Collectively, the evidence suggests that dual-marker screening using HbA1c and C-peptide offers a superior, physiologically grounded diagnostic framework capable of identifying at-risk individuals before overt diabetes develops. Integrating these biomarkers into clinical screening protocols could refine early intervention strategies and enable precision-based glycemic monitoring.

5. Conclusion and Recommendations

The present study underscores the combined diagnostic potential of HbA1c and C-peptide as complementary biomarkers for screening and stratifying diabetic and non-diabetic individuals. Our findings demonstrate that the integration of C-peptide, a marker of endogenous insulin secretion, with HbA1c, a measure of long-term glycemic control, significantly improves diagnostic accuracy and sensitivity. The combined model achieved an AUC of 0.92, outperforming either biomarker alone, confirming its reliability for early metabolic dysfunction detection.

Physiologically, this dual-marker approach provides a more nuanced understanding of β-cell activity and insulin resistance. Elevated C-peptide levels among prediabetic participants reflect compensatory β-cell responses, preceding the deterioration seen in overt diabetes. This aligns with recent global findings suggesting that early β-cell stress and hyperinsulinemia can be detected well before significant hyperglycemia manifests. Therefore, employing both biomarkers enables clinicians to detect metabolic dysregulation at an earlier, reversible stage.

From a clinical and public health perspective, the incorporation of C-peptide testing alongside HbA1c in routine screening protocols can improve risk stratification, particularly in ethnically diverse populations where HbA1c variability due to hemoglobinopathies is prevalent. Future longitudinal and multicentric studies are recommended to establish standardized cutoff values and evaluate cost-effectiveness in large-scale screening programs.

Ultimately, this study supports a paradigm shift toward dual-marker metabolic profiling for diabetes screening — a more physiologically accurate, sensitive, and preventive diagnostic strategy in the modern era of personalized medicine.

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Conflict of Interest Statement:

No conflicts of interest related to this study.

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