





# ORIGINAL ARTICLE

# Triglyceride-Glucose Index Versus Glycated Albumin: Diagnostic Tools for Early Detection of Insulin Resistance and Pre-Diabetes

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#### **ABSTRACT**

# Background:

Early detection of insulin resistance is essential for preventing type 2 diabetes and cardiovascular disease. Conventional methods like HOMA-IR rely on insulin assays, which may not be readily available in all clinical settings. This study explores the diagnostic potential of the Triglyceride-Glucose (TyG) Index and Glycated Albumin (GA) as accessible, non-insulinbased markers. Methods: A cross-sectional analysis was conducted on 120 adults aged 18-60 years. Anthropometric measurements and fasting blood samples were collected. Insulin resistance was determined using HOMA-IR, with a cut-off value >2.5. ROC analysis was performed to evaluate the predictive ability of TyG and GA, and multivariate regression was used to identify independent predictors. Results: Among the participants, 25% were classified as insulin resistant. The TvG Index showed a significant correlation with HOMA-IR (r = 0.26, p = 0.005), whereas GA did not (r = -0.12, p = 0.196). ROC analysis revealed a moderate predictive value for TyG (AUC = 0.691), while GA showed poor discrimination (AUC = 0.588). Multivariate analysis identified BMI and TyG as significant independent predictors of insulin resistance, with Glycated albumin contributing minimally. Conclusion: The TyG Index demonstrates promise as a practical, non-insulin-dependent marker for identifying insulin resistance, particularly in resource-limited settings. While Glycated Albumin remains useful for glycemic assessment, it may not reliably indicate insulin resistance in this population.

**Keywords:** Insulin resistance, Triglyceride-Glucose Index, Glycated Albumin, HOMA-IR, ROC curve, BMI, South Asian populations

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Received: 28 Apr 2025 Accepted: 08 Jun 2025

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#### 1. INTRODUCTION

Insulin resistance (IR) represents a fundamental pathophysiological process in the development of type 2 diabetes mellitus (T2DM), metabolic syndrome, and related cardiovascular complications [1,2]. It is characterized by a diminished response of target tissues to the physiological action of insulin, particularly in muscle, adipose tissue, and the liver [3]. Detecting insulin resistance early offers an opportunity for targeted interventions, lifestyle modifications, and risk stratification before overt hyperglycemia develops [4].

The standard clinical method to quantify insulin resistance is the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), which combines fasting plasma glucose and fasting insulin levels to estimate insulin sensitivity [5]. Despite its widespread use, HOMA-IR requires insulin assays, which are often not feasible in many primary care and resource-limited settings due to cost, standardization issues, and limited laboratory availability [6].

To address these challenges, alternative biomarkers that do not depend on insulin measurement have gained interest. The Triglyceride-Glucose (TyG) Index, calculated using fasting triglyceride and fasting glucose levels, has emerged as a promising and easily accessible surrogate marker for insulin resistance [7]. Several studies have reported its utility in various populations, including those at risk for diabetes and cardiovascular diseases [8].

Another proposed marker is Glycated Albumin (GA), which reflects short- to intermediate-term glycemic control over a period of 2 to 3 weeks. Although GA is often evaluated as an alternative to HbA1c in patients with hemoglobinopathies or altered red blood cell turnover, its association with insulin resistance remains unclear and varies across ethnic groups and metabolic profiles [9].

Given the growing burden of insulin resistance and type 2 diabetes in South Asian populations—who often present with higher cardiometabolic risk at lower BMI thresholds—there is a pressing need to evaluate simple, reliable, and cost-effective biomarkers. This study aims to assess and compare the diagnostic accuracy of the TyG Index and Glycated Albumin in identifying insulin resistance, using HOMA-IR as the reference standard, in a cohort of apparently healthy South Asian adults.

#### 2. MATERIALS AND METHODS

## **Study Design and Setting**

This six-month, cross-sectional observational study, conducted at Santiniketan Medical College and Hospital, West Bengal, India, aimed to compare the diagnostic utility of the Triglyceride-Glucose (TyG) Index and Glycated Albumin (GA) for insulin resistance, using HOMA-IR as the reference.

# **Ethical Considerations**

The Institutional Ethics Committee of Santiniketan Medical College and Hospital approved the study (Ref. No: SMC/ACAD/IEC/04112024). All participants provided written informed consent, adhering to the Declaration of Helsinki.

#### **Sample Size Estimation**

The required sample size was estimated using GPower version 3.1.9.7\*, assuming a medium effect size (Cohen's d = 0.5), significance level  $\alpha$  = 0.05, and power (1– $\beta$ ) = 0.80 for a two-tailed independent samples t-test comparing insulin-resistant and non-insulin-resistant groups. The calculated sample size was 102 participants. To accommodate potential exclusions and incomplete data, 120 participants were recruited. All 120 participants were included in the final analysis.

#### **Participant Selection**

Adults (18–60 years) undergoing metabolic risk evaluation were eligible. Exclusions included diagnosed diabetes, hepatic/renal dysfunction, inflammatory conditions, pregnancy, or hemoglobinopathies affecting GA. From 165 screened, 45 were excluded (28 ineligible, 12 declined, 5 incomplete data), resulting in 120 participants. (Figure 1 details recruitment).

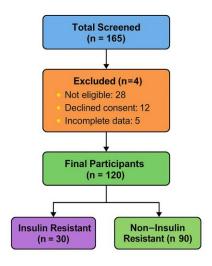


Figure 1. Recruitment of patients

#### **Clinical and Anthropometric Assessment**

Participants underwent demographic profiling, medical history, and anthropometric measurements (height, weight, waist/hip circumference) to derive BMI and waist-to-hip ratio. Blood pressure was also recorded. BMI categories for South Asian populations were applied: normal ( $<23.0 \text{ kg/m}^2$ ), overweight ( $23.0-24.9 \text{,kg/m}^2$ ), and obese ( $\ge 25.0 \text{ kg/m}^2$ ).

# **Biochemical Investigations**

Fasting venous blood samples (8–12 hour fast) were collected. Fasting plasma glucose and serum triglycerides were measured enzymatically. Fasting insulin was determined by ECLIA, and Glycated albumin by ELISA. Derived indices were calculated:

- TyG Index =  $\ln \left[ \frac{\text{Fasting triglycerides } (mg/dL) \times \text{Fasting glucose } (mg/dL)}{2} \right]$
- HOMA-IR =  $\frac{Fasting \ insulin \ (\mu IU/mL) \times Fasting \ glucose \ (mg/dL)}{405}$

Insulin resistance was defined as HOMA-IR > 2.5, based on South Asian population cut-offs.

# **Statistical Analysis**

Data were analyzed using SPSS v25.0. Means and percentages were used for descriptive statistics. Group comparisons used t-tests, ANOVA, or Chi-square tests. Correlation was assessed by Pearson's r. ROC analysis determined diagnostic accuracy. Multivariate regression identified predictors of HOMA-IR. Significance was set at p < 0.05.

## 3. RESULTS

#### **Baseline Clinical and Anthropometric Characteristics**

A total of 120 adult individuals, aged between 18 and 60 years, were enrolled to examine early metabolic indicators associated with insulin resistance. Key baseline data encompassing demographic, anthropometric, and clinical measures were collected and are presented in Table.1.

Table 1. Baseline Anthropometric and Clinical Profile of Participants (N = 120)

Variable	Mean ± SD or n (%)		
Age (years)	43.2 ± 10.3		
Sex			
Male	57 (47.5%)		
Female	63 (52.5%)		
Height (cm)	160.4 ± 8.0		
Weight (kg)	67.9 ± 11.8		
Body Mass Index (kg/m²)	26.1 ± 4.9		
Waist Circumference (cm)	88.4 ± 9.4		
Hip Circumference (cm)	95.2 ± 8.1		
Waist-to-Hip Ratio	0.92 ± 0.11		
Systolic Blood Pressure (mmHg)	137.4± 15.6		
Diastolic Blood Pressure (mmHg)	84.2 ± 8.1		
Family History of Diabetes			
Yes	45 (37.5%)		
No	75 (62.5%)		

Note: Continuous variables are expressed as mean ± standard deviation; categorical data are presented as number and percentage.

The participant pool included a slightly greater proportion of females than males. Nearly 38% reported a familial predisposition to diabetes. The average BMI indicated a predominance of overweight individuals, supported by high waist and hip circumference measurements suggesting central adiposity. Blood pressure readings were on the higher side of normal, aligning with pre-hypertensive profiles. These baseline metrics suggest that the study cohort possessed elevated metabolic risk, making them suitable for investigating non-insulin-based markers of insulin resistance such as the TyG Index and Glycated albumin.

## Comparison of Biochemical and Anthropometric Characteristics by Insulin Resistance Status

To evaluate metabolic disparities between individuals with and without insulin resistance, key clinical and biochemical metrics were assessed. Table 2 presents the comparative analysis between the insulin-resistant (IR) and non–insulin-resistant (non-IR) groups.

Table 2. Comparison of Biochemical and Anthropometric Characteristics Between IR and Non-IR Participants

Parameter	Non-IR (n = 90)	IR (n = 30)	p-value
Body Mass Index (kg/m²)	25.6 ± 4.7	27.5 ± 5.2	0.031
Fasting Plasma Glucose (mg/dL)	100.31 ± 12.91	107.44 ± 13.22	0.002
Fasting Triglycerides (mg/dL)	148.5 ± 36.2	162.7 ± 41.0	0.048
Fasting Insulin (μIU/mL)	7.06± 2.60	14.45 ± 3.14	< 0.001
HOMA-IR	1.74 ± 0.64	$3.81 \pm 0.69$	< 0.001
TyG Index	8.94 ± 0.30	9.06 ± 0.30	0.005
Glycated Albumin (%)	15.21 ± 1.52	14.63 ± 1.59	0.073

Note: Values are expressed as mean ± standard deviation. Independent t-tests were applied for between-group comparisons. Significance set at p < 0.05.

Participants, categorized as insulin-resistant based on HOMA-IR > 2.5, exhibited significantly higher BMI and triglyceride levels compared to non-IR individuals. Additionally, plasma glucose and fasting insulin concentrations were substantially elevated in the IR group, which also reflected in higher TyG Index scores (p = 0.005). While Glycated Albumin values showed a slight reduction in the IR group, the difference did not reach statistical significance. These observations reinforce the metabolic distinctions between the two groups, particularly in lipid and glycemic indices.

#### Correlation Between Biochemical Markers and HOMA-IR

To explore the strength and direction of associations between insulin resistance and individual metabolic indicators, Pearson's correlation analysis was conducted using HOMA-IR as the reference variable. The outcomes are summarized in Table 3.

Table 3. Correlation of Metabolic Parameters with HOMA-IR (n = 120)

Parameter	Correlation Coefficient (r)	95% Confidence Interval	p-value
Fasting Insulin (μIU/mL)	0.95	0.93-0.97	<0.001
Fasting plasma glucose (mg/dL)	0.44	0.30 - 0.56	< 0.001
Fasting Triglycerides (mg/dL)	0.32	0.15-0.47	< 0.001
TyG Index	0.26	0.08 - 0.42	0.005
Glycated Albumin (%)	-0.12	-0.30 to 0.06	0.196

Pearson's correlation was used to evaluate linear associations between individual biomarkers and HOMA-IR. p < 0.05 was considered statistically significant.

Among the parameters examined, fasting insulin displayed the strongest positive correlation with HOMA-IR (r = 0.95), consistent with its central role in the formula for this index. Fasting glucose (r = 0.44) and triglycerides (r = 0.32) also demonstrated significant positive correlations. The TyG Index, while less strongly correlated, still showed a statistically meaningful association (r = 0.26, p = 0.005), supporting its potential use as a surrogate marker for insulin resistance. Conversely, Glycated Albumin exhibited a weak negative correlation that was not statistically significant, suggesting limited relevance to HOMA-IR in this cohort.

# Evaluation of the Diagnostic Value of TyG Index and Glycated Albumin

The diagnostic accuracy of the Triglyceride-Glucose (TyG) Index and Glycated Albumin (GA) in detecting insulin resistance was assessed using Receiver Operating Characteristic (ROC) curve analysis, with HOMA-IR > 2.5 set as the benchmark. Key metrics such as the area under the ROC curve (AUC), optimal thresholds determined via Youden's Index, sensitivity, specificity, and overall classification performance are summarized in Table 4. Corresponding ROC curves are illustrated in Figure 2.

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Table 4. Diagnostic Performance of TyG Index and Glycated Albumin in Detecting Insulin Resistance (HOMA-IR > 2.5)

Biomarker	AUC (95% CI)	Optimal Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	Youden's Index
TyG Index	0.691 (0.589-0.776)	9.02	70.0% (50.6-85.3)	62.2% (51.3-72.3)	0.322
Glycated Albumin (%)	0.588 (0.481-0.690)	14.8	53.3% (34.3-71.7)	56.7% (45.8-67.1)	0.100

Abbreviations: AUC - Area Under the Curve; CI - Confidence Interval; ROC - Receiver Operating Characteristic.

As illustrated in Figure 2, the TyG Index achieved a fair discriminatory performance with an AUC of 0.691. The optimal cut-off value of 9.02 provided a sensitivity of 70.0% and specificity of 62.2%, indicating a balanced ability to identify true positives and minimize false positives. In comparison, Glycated Albumin demonstrated limited diagnostic strength, with an AUC of 0.588, and lower sensitivity (53.3%) and specificity (56.7%) at the cut-off point of 14.8%. These results underscore the TyG Index as a feasible surrogate marker for insulin resistance, especially in clinical settings where insulin measurement may not be routinely available. Although it offers only moderate predictive accuracy, its performance is superior to that of Glycated albumin in this South Asian cohort, reinforcing its potential role in metabolic risk screening.

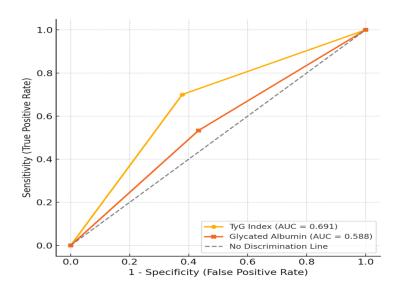


Figure 2. ROC Curves for TyG Index and Glycated Albumin

# Correlation of TyG Index and Glycated Albumin with HOMA-IR

To evaluate the strength of association between insulin resistance and non–insulin-based surrogate markers, Pearson correlation analysis was performed using HOMA-IR as the reference variable. The correlation coefficients, 95% confidence intervals, and p-values are presented in Table 3.

Table 3. Correlation of Biochemical Parameters with HOMA-IR (n = 120).

Parameter	Correlation Coefficient (r)	95% Confidence Interval	p-value
Fasting Insulin (μIU/mL)	0.95	0.93-0.97	<0.001
Fasting plasma glucose (mg/dL)	0.44	0.30 – 0.56	< 0.001
Fasting Triglycerides (mg/dL)	0.32	0.15-0.47	< 0.001
TyG Index	0.26	0.08 - 0.42	0.005
Glycated Albumin (%)	-0.12	-0.30 to 0.06	0.196

 $Correlation\ coefficients\ were\ calculated\ using\ Pearson's\ method.\ A\ p-value < 0.05\ was\ considered\ statistically\ significant.$ 

The TyG Index showed a weak but statistically significant positive correlation with HOMA-IR (r = 0.26, p = 0.005), suggesting its potential as a modest surrogate marker for insulin resistance. In contrast, Glycated albumin exhibited a weak and statistically non-significant negative correlation (r = -0.12, p = 0.196), indicating limited clinical utility for predicting insulin resistance. These associations are visually illustrated in Figure 3. The scatter plot for the TyG Index (Figure 3a) shows a mild upward trend with a defined regression line and 95% confidence band, consistent with the statistically significant correlation observed. Conversely, the scatter plot for Glycated Albumin (Figure 3b) reveals a diffuse distribution with no apparent trend, reinforcing the lack of meaningful correlation with HOMA-IR.

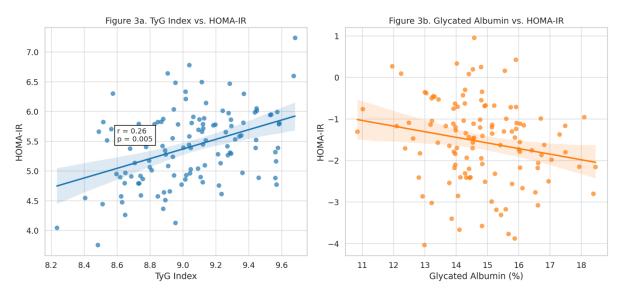


Figure 3. Correlation Between Biomarkers and HOMA-IR. Figure 3a. showing TyG Index vs. HOMA-IR with regression line and 95% confidence interval. Figure 3b. showing Glycated Albumin (%) vs. HOMA-IR with regression line and 95% confidence interval.

# Subgroup Analysis by Body Mass Index (BMI)

To evaluate the influence of adiposity on insulin resistance markers, participants were categorized into three BMI-based groups using the Asia-Pacific classification by the World Health Organization: normal weight (18.5–22.9 kg/m²), overweight (23.0–24.9 kg/m²), and obese ( $\geq$ 25.0 kg/m²). Each group included 40 individuals (n = 40), facilitating balanced intergroup comparisons. The mean levels of HOMA-IR, TyG Index, and Glycated Albumin (GA) across these subgroups are shown in Table 5.

Table 5. Comparison of	f Insulin Resistance	Markers Across BMI	Categories ( $n = 120$ ).
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Parameter	Normal (n = 40)	Overweight (n = 40)	Obese (n = 40)	p-value
HOMA-IR	1.78 ± 0.42	2.61 ± 0.54	$3.39 \pm 0.63$	< 0.001
TyG Index	8.74 ± 0.27	9.13 ± 0.21	9.32 ± 0.38	0.002
Glycated Albumin (%)	14.53 ± 1.07	14.98± 1.01	14.79 ± 1.29	0.341

Values are expressed as mean ± standard deviation. One-way ANOVA was used for group comparisons. A p-value < 0.05 was considered statistically significant.

There was a clear and statistically significant trend of increasing HOMA-IR with rising BMI (p < 0.001), underscoring a strong relationship between body fat accumulation and insulin resistance. A similar pattern was noted for the TyG Index (p = 0.002), further supporting its potential as a non-insulin-based proxy for metabolic risk. On the other hand, GA levels remained relatively stable across all BMI categories (p = 0.341), indicating its limited responsiveness to changes in body composition within this cohort.

## Multivariate Regression Analysis for Determinants of Insulin Resistance

A multivariate linear regression model was developed to explore the independent associations of select metabolic markers with insulin resistance, operationalized using HOMA-IR as the outcome variable. The predictors incorporated into the model included Body Mass

Index (BMI), Triglyceride-Glucose (TyG) Index, Fasting Plasma Glucose (FPG), and Glycated Albumin (GA). These variables were selected based on clinical significance and prior univariate associations.

The resulting model demonstrated strong explanatory power, accounting for 58% of the variance in HOMA-IR ( $R^2 = 0.58$ , F(4,115) = 39.84, p < 0.001). Detailed regression estimates—including unstandardized coefficients, standard errors, and standardized beta values—are presented in Table 6.

Predictor	B (Coefficient)	Standard Error (SE)	Standardized β	t-value	p-value
Body Mass Index (kg/m²)	0.12	0.03	0.31	4.00	<0.001
TyG Index	0.62	0.14	0.28	4.43	< 0.001
Fasting Plasma Glucose (mg/dL)	0.01	0.004	0.18	2.50	0.014
Glycated Albumin (%)	-0.03	0.05	-0.06	-0.60	0.550

Table 6. Multivariate Regression Analysis Predicting HOMA-IR (n = 120).

Model summary: R2 = 0.58, F(4,115) = 39.84, p < 0.001. All variance inflation factor (VIF) values < 2.5, confirming absence of multicollinearity.

From the regression output, both BMI and the TyG Index emerged as statistically significant predictors of insulin resistance, with standardized  $\beta$  values of 0.31 and 0.28, respectively (both p < 0.001). Fasting plasma glucose also showed a modest but meaningful association ( $\beta$  = 0.18, p = 0.014). Glycated Albumin, however, did not demonstrate a significant predictive relationship with HOMA-IR ( $\beta$  = -0.06, p = 0.550), suggesting its limited utility in this context. These results underscore the clinical utility of BMI and the TyG Index as non-insulin-based metrics that can effectively flag elevated insulin resistance risk. In contrast, GA may serve better as a glycemic marker than as an insulin resistance surrogate in this population.

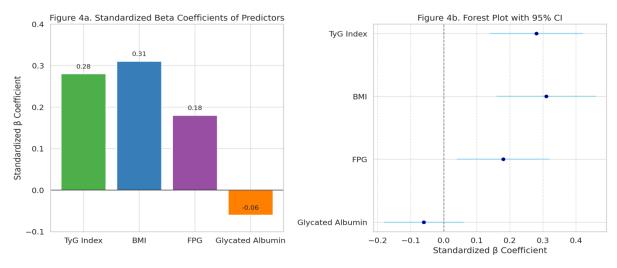


Figure 4. Multivariate Predictors of HOMA-IR. Figure 4a presents a bar chart of standardized  $\beta$  coefficients, illustrating the relative strength of each predictor in the regression model. Figure 4b provides a forest plot of the same predictors, including 95% confidence intervals. The plot shows that the effects of BMI and TyG Index were both statistically significant (CIs not crossing zero), while Glycated Albumin's confidence interval includes zero, confirming its lack of statistical contribution.

# 4. DISCUSSION

This study explored the effectiveness of the Triglyceride-Glucose (TyG) Index and Glycated Albumin (GA) as potential indicators of insulin resistance (IR), using the HOMA-IR > 2.5 threshold as a reference standard. Among the 120 participants analyzed, a substantial proportion exhibited elevated metabolic risk, as reflected by their BMI, waist circumference, and blood pressure profiles. These characteristics align with existing evidence that central obesity and hypertension are key contributors to insulin resistance in South Asian populations [10].

The comparison between insulin-resistant and non-insulin-resistant individuals revealed that the IR group had significantly higher fasting glucose, insulin, triglyceride levels, and BMI. These findings are consistent with prior studies indicating that visceral adiposity and dyslipidemia are strongly linked with insulin resistance [11]. The observed elevation in HOMA-IR and TyG Index values among the IR group further supports the utility of these indices in metabolic risk stratification.

When evaluating the correlation between biomarkers and HOMA-IR, fasting insulin unsurprisingly emerged as the strongest correlate, given its direct role in HOMA-IR calculation. Fasting plasma glucose and triglycerides also showed moderate positive correlations, reinforcing their utility in indirect IR assessment. The TyG Index, though weaker in correlation strength, still showed a statistically significant association with HOMA-IR, lending support to its use in low-resource settings where insulin assays may not be feasible. In contrast, Glycated Albumin did not show a significant association with HOMA-IR, possibly due to its higher sensitivity to glycemic variability and shorter monitoring window compared to glycated hemoglobin.

The ROC curve analysis demonstrated that the TyG Index had fair discriminatory capacity (AUC = 0.691), with acceptable sensitivity and specificity at the defined cut-off. This performance indicates moderate diagnostic accuracy, particularly useful in large-scale screening or field-based interventions where conventional IR markers like fasting insulin may be impractical. Although the diagnostic value of Glycated albumin was inferior (AUC = 0.588), its non-invasive nature and rapid turnover may still justify its use in complementary contexts [12].

Further subgroup analysis by BMI highlighted a trend of increasing HOMA-IR and TyG Index values with rising adiposity levels. This association emphasizes the well-established pathophysiological role of excess adipose tissue in promoting insulin resistance via pro-inflammatory and lipotoxic pathways. Interestingly, Glycated albumin levels did not vary significantly across BMI categories, indicating its limited responsiveness to adiposity-related IR changes [13].

Multivariate regression modeling revealed that BMI and TyG Index were the most robust independent predictors of HOMA-IR, surpassing fasting plasma glucose and GA in predictive strength. This reinforces the emerging perspective that integrated lipid-glucose metrics such as TyG Index capture multiple dimensions of metabolic dysfunction and may outperform single glycemic markers in IR prediction[14].

Overall, these findings underscore the potential of the TyG Index as a practical and accessible surrogate marker for insulin resistance in resource-limited settings, particularly among high-risk populations such as South Asians. The limited utility of Glycated Albumin in this context suggests that its role may be more suited to monitoring glycemic control rather than identifying insulin resistance per se.

# Limitations

This study has several limitations that warrant consideration. First, its cross-sectional design precludes the establishment of causal relationships between metabolic markers and insulin resistance. While associations were observed, temporal sequencing could not be assessed. Second, although multivariate analysis was performed, the potential influence of unmeasured confounding factors such as dietary habits, physical activity levels, and genetic predisposition could not be fully excluded. Third, the relatively small sample size and single-center recruitment may limit the generalizability of findings to broader South Asian populations. Future prospective, multicenter studies are needed to validate these results and establish temporal and causal inferences.

#### 5. CONCLUSION

The TyG Index emerged as a meaningful, affordable, and readily accessible marker for insulin resistance in this South Asian cohort. It demonstrated better diagnostic accuracy than Glycated Albumin and maintained statistical significance in multivariate analyses. The incorporation of TyG Index into routine assessments, especially when paired with BMI, may enhance early detection and risk stratification for insulin resistance in high-risk populations. Glycated albumin, although useful in specific clinical contexts, appears to have limited utility for IR screening in normoglycaemic individuals. Further studies with larger sample sizes and prospective designs are warranted to validate these findings and explore their applicability across diverse demographic settings.

**Ethics Approval and Consent to Participate:** This study was approved by the Institutional Ethics Committee of Santiniketan Medical College and Hospital, West Bengal, India (Ref. No: SMC/ACAD/IEC/04112024). All participants provided written informed consent prior to participation, in accordance with the Declaration of Helsinki.

**Consent for Publication:** All authors have reviewed the final manuscript and consent to its publication in Batna Journal of Medical Sciences.

**Availability of Data and Materials:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Competing Interests:** The authors declare that they have no competing interests.

Funding: This study received no external funding. It was conducted as an investigator-initiated academic research project.

Authors' Contributions: Dr. Sandip Kumar Kundu: conceptualization, methodology, supervision; Dr. Kiran Gupta: Biochemical Analysis, Data Curation, Literature Review; Dr. Subhasis Mukherjee: Patient Recruitment, Data Collection; Dr. Sandip Ghosh (Corresponding Author): Formal Analysis, Manuscript Drafting, Revision, Project Administration; Dr. Arbind Kumar Choudhary: Statistical Analysis, Interpretation of Results, Proofreading; Dr. Kamala Kanta Parhi: Graphical Representation, Figure Preparation, Final Review. All authors have read and approved the final version of the manuscript.

**Acknowledgements:** The authors sincerely thank the laboratory and technical staff of Santiniketan Medical College and IQ City Medical College for their support during sample processing and analysis.

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