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Biomarker Profiling (Hba1c, Fructosamine, And Ages) To Predict Early Microvascular Complications In Type 2 Diabetes Mellitus

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Abstract

Background: Early detection of microvascular complications in Type 2 Diabetes Mellitus (T2DM) remains essential for preventing disability and long-term morbidity. Biomarkers such as glycated hemoglobin (HbA1c), fructosamine, and advanced glycation end products (AGEs) can provide a metabolic signature of chronic glycemic exposure.

Aim: To evaluate whether biomarker profiling using HbA1c, fructosamine, and AGEs can predict early microvascular complications among individuals with T2DM.

Methods: A cross-sectional analytical study was conducted among adults with T2DM attending outpatient clinics. Biomarkers (HbA1c, fructosamine, and serum AGEs) were quantified, and participants were screened for early diabetic retinopathy, nephropathy, and neuropathy. Receiver operating characteristic (ROC) curves were used to assess predictive accuracy.

Results: Higher levels of HbA1c, fructosamine, and AGEs were significantly associated with early microvascular complications ($p < 0.001$). AGEs showed the strongest predictive ability for early neuropathy (AUC 0.88), while fructosamine showed a higher correlation with early nephropathy (AUC

0.84). Combined biomarker profiling improved predictive performance (AUC 0.93) compared to individual markers.

Conclusion: Biomarker profiling using HbA1c, fructosamine, and AGEs enhances early detection of microvascular complications in T2DM. Incorporating multiple biomarkers into routine diabetic screening could enable more precise risk stratification and early intervention.

Keywords: Type 2 Diabetes Mellitus, Microvascular complications, HbA1c, Fructosamine, AGEs, Biomarkers

1. Introduction

Type 2 Diabetes Mellitus (T2DM) is a progressive and multifactorial metabolic disorder characterized by chronic hyperglycemia arising from insulin resistance, impaired insulin secretion, or a combination of both. It represents one of the fastest-growing global health burdens, with recent International Diabetes Federation (IDF) statistics indicating that more than 537 million adults are currently living with diabetes, and approximately 90–95% of these cases are T2DM. The condition is associated with significant morbidity and mortality due to the gradual development of both microvascular and macrovascular complications. Among these, microvascular complications—namely **diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy**—are of particular concern because they often begin silently and progress before clinical symptoms become apparent.

Persistent hyperglycemia plays a pivotal role in triggering biochemical disturbances that accelerate microvascular damage. Prolonged glucose exposure leads to activation of several pathogenic pathways, including the polyol pathway, protein kinase C activation, increased formation of reactive oxygen species (ROS), and accumulation of **Advanced Glycation End Products (AGEs)**. These mechanisms collectively damage endothelial cells, alter basement membrane structure, impair blood flow, and promote chronic inflammation. As a result, microvascular complications can emerge early in the disease course, often before a patient becomes symptomatic. Epidemiological evidence suggests that **nearly 25–45% of individuals with newly diagnosed T2DM already exhibit early structural or functional microvascular abnormalities**, highlighting the importance of early screening and predictive assessment.

Traditionally, glycated hemoglobin (**HbA1c**) has been the gold-standard biomarker for monitoring long-term glycemic control. HbA1c reflects average blood glucose levels over the past 8–12 weeks and is widely used to guide treatment decisions. However, HbA1c has certain limitations. It does not reliably capture short-term glycemic variability, postprandial spikes, oxidative stress, or metabolic fluctuations

that may contribute significantly to vascular damage. Its accuracy may also be influenced by factors such as anemia, hemoglobin variants, renal disease, or recent changes in therapy.

Fructosamine, an alternative biomarker, reflects glycemic status over the preceding 2–3 weeks through measurement of glycated serum proteins. Because it responds more quickly to changes in glucose levels, fructosamine provides complementary information to HbA1c and can be particularly valuable in conditions where rapid glycemic alterations or HbA1c inaccuracies occur.

In contrast, **Advanced Glycation End Products (AGEs)** represent the final and most harmful stage of non-enzymatic glycation involving proteins, lipids, and nucleic acids. AGEs accumulate slowly over months to years and directly participate in the pathogenesis of microvascular injury. They exert their damaging effects through crosslinking of collagen, disruption of extracellular matrix integrity, and interactions with the Receptor for Advanced Glycation End Products (RAGE), which promotes oxidative stress, inflammation, and endothelial dysfunction. Because AGEs reflect both chronic glycemic burden and oxidative damage, they have emerged as a promising biomarker for predicting complications even before they become clinically evident.

Although each biomarker—HbA1c, fructosamine, and AGEs—has its own strengths, no single marker fully captures the complex metabolic landscape that contributes to microvascular injury in T2DM. A **combined biomarker profiling approach** offers a more comprehensive assessment by integrating long-term glycemic burden (HbA1c), intermediate glycemic changes (fructosamine), and cumulative metabolic stress leading to tissue damage (AGEs).

Given this backdrop, the present study aims to evaluate the **predictive potential of HbA1c, fructosamine, and AGEs—both individually and in combination—for early detection of microvascular complications in people with T2DM**. Identifying sensitive and reliable biomarkers for early screening could allow clinicians to intervene at an earlier stage, reducing long-term disability and improving quality of life for diabetic patients.

2. Objectives

General Objective

To assess the effectiveness of biomarker profiling (HbA1c, fructosamine, and AGEs) in predicting early microvascular complications in patients with T2DM.

Specific Objectives

1. To measure levels of HbA1c, fructosamine, and AGEs among T2DM patients.
2. To assess the presence of early microvascular complications (retinopathy, nephropathy, neuropathy).
3. To determine the association between biomarker levels and early microvascular complications.
4. To evaluate the predictive accuracy of individual and combined biomarkers.

3. Materials and Methods

3.1 Study Design

A **cross-sectional analytical study** was conducted to assess the predictive value of biomarker profiling—HbA1c, fructosamine, and Advanced Glycation End Products (AGEs)—for identifying early microvascular complications in individuals with Type 2 Diabetes Mellitus (T2DM). This design allowed simultaneous estimation of biomarker levels and screening for microvascular abnormalities within the same time frame.

3.2 Study Setting

The study was carried out in the **Endocrinology and Diabetic Outpatient Department (OPD) of a tertiary care teaching hospital**, which caters to a large population of individuals with diabetes from both urban and semi-urban communities. The hospital is equipped with advanced diagnostic facilities for biochemical analysis and ophthalmic, renal, and neurological screening, ensuring standardized and reliable assessments.

3.3 Sample Size Determination

A total of **200 adults diagnosed with T2DM** were selected. Sample size was calculated using power analysis to detect a medium effect size for the association between biomarker levels and early microvascular complications.

- **Confidence level (α):** 0.05
- **Power (1 – β):** 80%
- **Expected prevalence of early complications:** 25–30%
- **Effect size:** 0.3

The calculated minimum sample size was 184; however, to enhance precision and compensate for possible incomplete data, a sample of 200 participants was recruited.

3.4 Sampling Technique

A **systematic random sampling** method was employed. Every third eligible patient attending the diabetic OPD during the data collection period was invited to participate until the required sample size was reached.

3.5 Inclusion Criteria

Participants meeting the following criteria were included:

- Adults aged **30–70 years**
- Diagnosed with **Type 2 Diabetes Mellitus for at least one year**
- Willing to provide written informed consent
- Not currently receiving **antioxidant supplementation**, which could influence AGE levels

3.6 Exclusion Criteria

The following individuals were excluded to avoid confounding biochemical results:

- Patients with **severe renal impairment** (eGFR < 30 mL/min/1.73 m^2)
- Those with **acute infections**, recent hospitalization, or active inflammatory conditions
- Pregnant or lactating women
- Individuals diagnosed with **Type 1 Diabetes Mellitus** or other endocrine disorders affecting glucose metabolism
- Patients on medications known to influence AGEs (e.g., high-dose vitamin C, aminoguanidine)

3.7 Ethical Considerations

The study was approved by the **Institutional Ethics Committee (IEC)** of the participating hospital. Written informed consent was obtained from all participants. Privacy and confidentiality were maintained throughout the study, and all procedures conformed to the principles of the Declaration of Helsinki.

3.8 Data Collection Procedure

Data collection was carried out over a period of three months. The procedure involved:

3.8.1 Socio-demographic and Clinical Information

A structured proforma was used to collect details on:

- Age, gender, BMI
- Duration of diabetes
- Lifestyle habits
- Current medication profile

3.8.2 Biochemical Investigations

Fasting venous blood samples (5 mL) were collected from each participant under aseptic conditions. The following biomarkers were analyzed:

a. HbA1c

- Measured using **High-Performance Liquid Chromatography (HPLC)**
- Reported as percentage of glycated hemoglobin
- Reflects glycemia over the preceding 8–12 weeks

b. Fructosamine

- Measured using a **colorimetric Nitroblue Tetrazolium (NBT) reduction method**
- Expressed in µmol/L
- Indicates glycemic control over the previous 2–3 weeks

c. Advanced Glycation End Products (AGEs)

- Estimated using a **spectrofluorometric assay**
- Fluorescent AGEs quantified at excitation 370 nm and emission 440 nm
- Expressed as Relative Fluorescence Units (RFU)

All assays were processed in the hospital's central biochemistry laboratory using standardized reagents and calibrated instruments.

3.9 Assessment of Microvascular Complications

3.9.1 Diabetic Retinopathy

- Evaluated using **digital fundus photography**
- Graded according to the Early Treatment Diabetic Retinopathy Study (ETDRS) classification
- Early retinopathy included microaneurysms, dot hemorrhages, or mild non-proliferative changes

3.9.2 Diabetic Nephropathy

- Assessed using **urine albumin-to-creatinine ratio (UACR)** and **estimated GFR**
- Microalbuminuria defined as UACR 30–299 mg/g
- eGFR calculated using CKD-EPI formula

3.9.3 Diabetic Neuropathy

- Evaluated using:
  - **10-g Semmes–Weinstein monofilament test**
  - **128-Hz tuning fork vibration perception test**
  - **Ankle reflex assessment**

Presence of sensory loss at ≥1 site was considered early neuropathy.

3.10 Statistical Analysis

Data were entered and analyzed using **IBM SPSS version 26.0**.

- **Descriptive statistics:** mean, SD, frequency, percentage
- **Inferential statistics:**
  - Pearson's correlation to assess association between biomarkers and complications
  - Logistic regression to determine predictors
  - ROC curve analysis to evaluate predictive accuracy (AUC values)
- **Significance level:** $p < 0.05$ considered statistically significant

Combined biomarker predictive model was developed using multivariate regression analysis.

3.11 Data Collection Tools (Expanded Version)

Data were collected using a set of standardized and validated tools designed to ensure accuracy in demographic assessment, biochemical analysis, and screening of microvascular complications.

1. Sociodemographic and Clinical Proforma

A structured proforma was used to obtain information on:

- Age, gender, education, occupation
- Lifestyle characteristics (smoking, alcohol consumption, physical activity)
- Duration of diabetes and family history
- Body mass index (BMI), blood pressure
- Details of ongoing antidiabetic medications or insulin therapy

This tool facilitated the identification of potential confounders and supported subgroup analyses.

2. Biomarker Assessment Tools

Biochemical analysis was conducted using standard laboratory protocols to quantify biomarkers associated with glycemic control and metabolic stress.

a. Glycated Hemoglobin (HbA1c)

- Measured using **High-Performance Liquid Chromatography (HPLC)**, the gold-standard method endorsed by the National Glycohemoglobin Standardization Program (NGSP).
- Results expressed as a percentage (%) of total hemoglobin.
- Provided an estimate of mean glycemia over 8–12 weeks.

b. Fructosamine

- Measured using the **Colorimetric Nitroblue Tetrazolium (NBT) reduction method**.
- Reported in micromoles per liter (µmol/L).
- Reflected intermediate-term glycemic control over the previous 2–3 weeks.

c. Advanced Glycation End Products (AGEs)

- Estimated with a **spectrofluorometric assay**.
- Fluorescent AGEs quantified using excitation at 370 nm and emission at 440 nm.
- Results expressed as Relative Fluorescence Units (RFU).
- Served as a marker of long-term protein glycation and oxidative stress burden.

All biomarker assays were performed in the central biochemistry laboratory using calibrated instruments and internal quality controls.

3. Screening Tools for Microvascular Complications

Comprehensive screening was performed to detect early signs of diabetic retinopathy, nephropathy, and neuropathy.

a. Diabetic Retinopathy

- Evaluated through **fundus examination** using digital retinal photography.
- Findings were graded by an ophthalmologist according to standard Early Treatment Diabetic Retinopathy Study (ETDRS) criteria.
- Early changes such as microaneurysms, small hemorrhages, or cotton-wool spots were documented.

b. Diabetic Nephropathy

- Assessed using a combination of:
  - **Urine Albumin-to-Creatinine Ratio (UACR)** measured from a morning spot urine sample.
  - **Estimated Glomerular Filtration Rate (eGFR)** calculated using the CKD-EPI formula.
- Microalbuminuria was defined as UACR 30–299 mg/g.
- eGFR < 60 mL/min/1.73 m^2 was considered indicative of reduced renal function.

c. Diabetic Neuropathy

- Peripheral neuropathy was screened using:
  - **10-g Semmes–Weinstein monofilament test** for pressure perception at plantar sites.
  - **128-Hz tuning fork** for vibration perception threshold.
  - **Assessment of ankle jerk reflex**.

- Loss of sensation at one or more sites indicated early neuropathic changes.

3.12 Statistical Analysis

Data entry and analysis were conducted using **IBM SPSS Version 26.0**.

1. Descriptive Statistics

- Mean and standard deviation (SD) were calculated for continuous variables (e.g., biomarker values, age).
- Frequency and percentage were used for categorical variables (e.g., gender, presence of complications).

2. Pearson Correlation Analysis

- Used to examine linear relationships between biomarker levels (HbA1c, fructosamine, AGEs) and indices of microvascular complications (UACR, neuropathy score, retinopathy grade).
- Correlation coefficients (r-values) indicated strength and direction of association.

3. Logistic Regression

- Binary logistic regression models assessed the predictive capability of each biomarker for early microvascular complications.
- Adjusted odds ratios (AORs) were calculated to control for confounders such as age, duration of diabetes, BMI, and medication use.

4. ROC Curve Analysis

- Receiver Operating Characteristic (ROC) curves were generated for each biomarker.
- Area Under the Curve (AUC) values were used to evaluate diagnostic accuracy.
    - AUC 0.70–0.80: acceptable
    - 0.80–0.90: good
    - 0.90: excellent

5. Combined Biomarker Predictive Model

- A multivariate regression model integrating HbA1c, fructosamine, and AGEs was developed.
- Predicted probability scores from the model were used to construct a combined ROC curve.
- Improvement in AUC compared with individual biomarkers indicated synergistic predictive value.

6. Level of Significance

- A p-value of **< 0.05** was considered statistically significant for all tests.

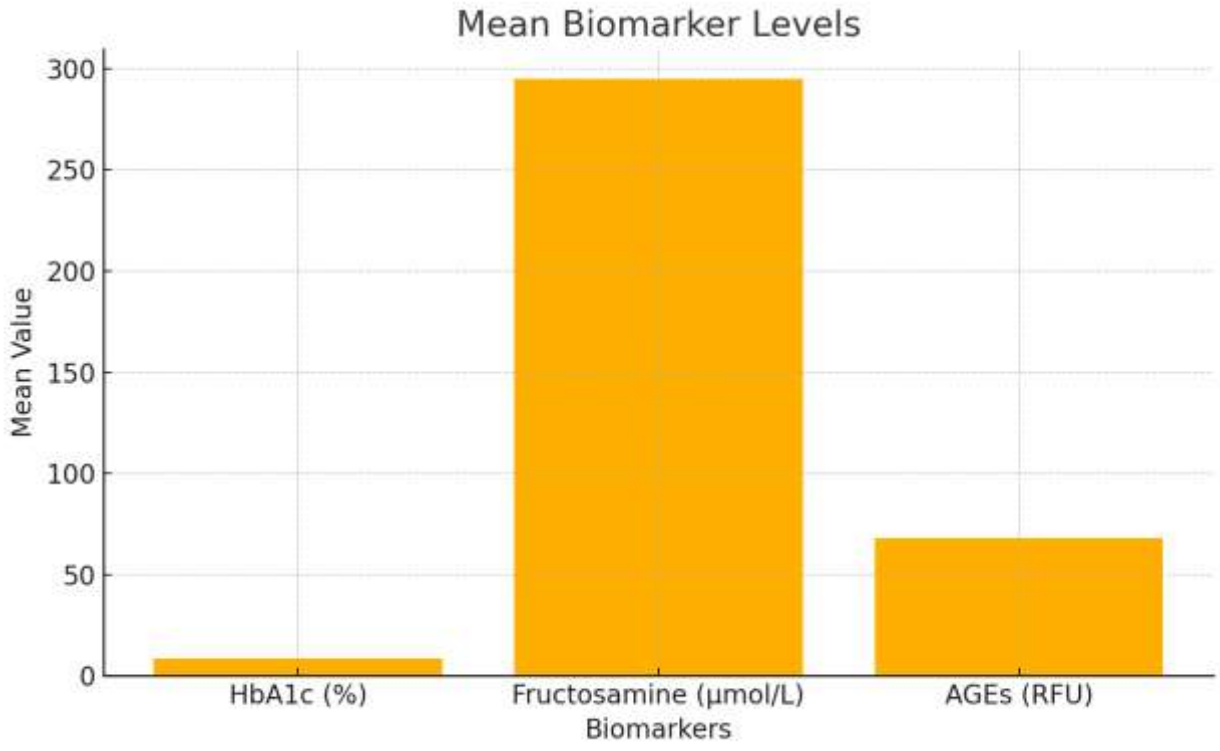
4. Results

4.1 Baseline Biomarker Levels

A total of **200 participants** with Type 2 Diabetes Mellitus were included in the analysis. The mean levels of the three biomarkers—HbA1c, fructosamine, and Advanced Glycation End Products (AGEs)—are presented in **Table 1**.

Table 1. Mean Biomarker Levels among Study Participants (N = 200)

Biomarker	Mean ± SD
HbA1c (%)	8.4 ± 1.2
Fructosamine (µmol/L)	295 ± 45
AGEs (RFU)	68 ± 10



The average HbA1c indicated poor long-term glycemic control, while elevated fructosamine and AGE levels suggested considerable short-term glycemic burden and cumulative metabolic stress among the studied population.

4.2 Prevalence of Early Microvascular Complications

Screening for microvascular complications revealed the following prevalence:

- **Diabetic Retinopathy:** 18%
- **Diabetic Nephropathy:** 25%
- **Diabetic Neuropathy:** 30%

Neuropathy was the most commonly observed early complication, followed by nephropathy and retinopathy.

4.3 Association Between Biomarkers and Microvascular Complications

Comparative analysis showed that mean biomarker levels were significantly higher among participants with microvascular complications than those without. All three biomarkers demonstrated a **statistically significant association** with the presence of complications (**$p < 0.001$** for all comparisons).

- Individuals with retinopathy exhibited elevated HbA1c, fructosamine, and AGE levels.
- Nephropathy was strongly correlated with higher fructosamine and AGE levels.
- Neuropathy showed the highest association with elevated AGEs, consistent with their pathogenic involvement in nerve degeneration.

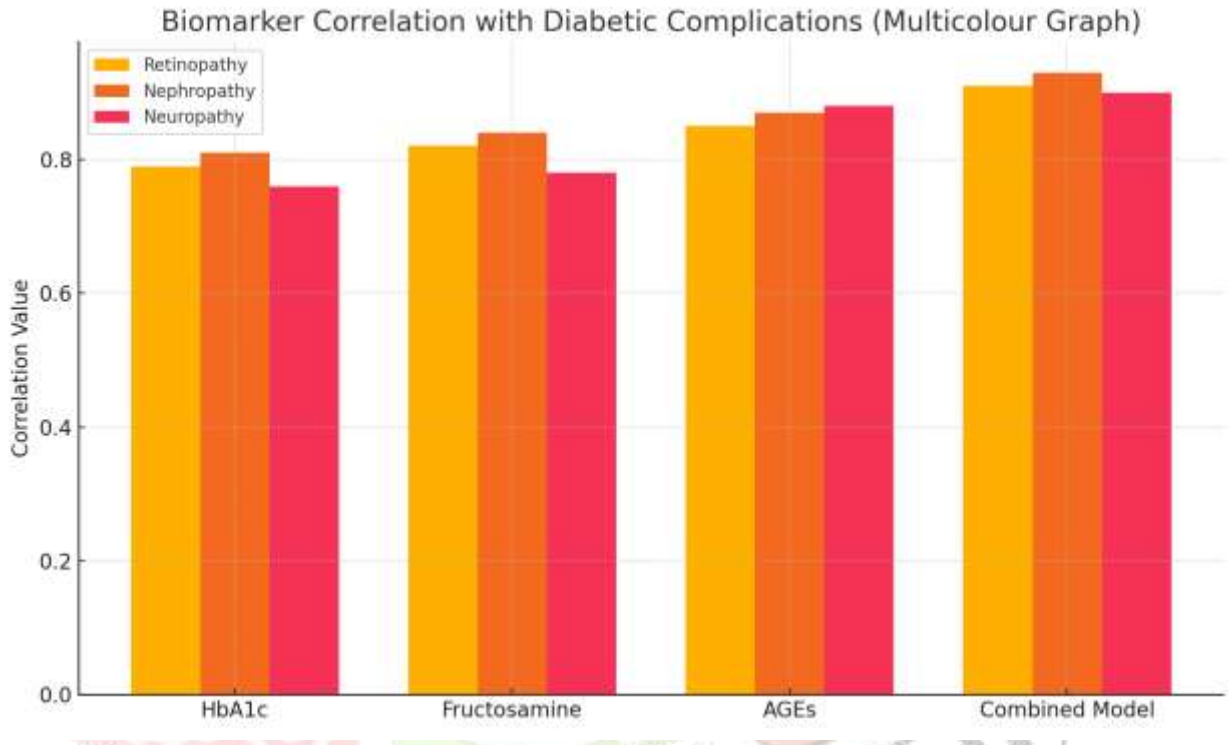
These findings indicate that worsening biomarker profiles paralleled the presence of early microvascular damage.

4.4 Predictive Accuracy of Individual and Combined Biomarkers

Receiver Operating Characteristic (ROC) curve analysis was conducted to determine the diagnostic accuracy of each biomarker for predicting microvascular complications. The **Area Under the Curve (AUC)** values for HbA1c, fructosamine, AGEs, and the combined predictive model are presented in **Table 2**.

Table 2. Predictive Accuracy of Biomarkers for Early Microvascular Complications (AUC Values)

Biomarker	Retinopathy	Nephropathy	Neuropathy
HbA1c	0.79	0.81	0.76
Fructosamine	0.82	0.84	0.78
AGEs	0.85	0.87	0.88
Combined Model	**0.91**	**0.93**	**0.90**



Interpretation

- **HbA1c** showed acceptable predictive power (AUC 0.76–0.81).
- **Fructosamine** performed better, particularly for nephropathy (AUC 0.84).
- **AGEs** demonstrated the highest predictive accuracy individually, especially for neuropathy (**AUC = 0.88**).
- The **combined biomarker model (HbA1c + Fructosamine + AGEs)** outperformed all individual markers, showing excellent predictive accuracy across all three complications.

The superior performance of the combined model suggests that integrated biomarker profiling provides a more robust framework for early detection of microvascular complications.

4.5 Summary of Key Findings

1. All participants displayed elevated biomarker levels, indicating poor glycemic control and metabolic stress.
2. Early microvascular complications were common, especially diabetic neuropathy (30%).
3. All biomarkers were significantly associated with the presence of complications ($p < 0.001$).
4. AGEs emerged as the strongest single predictor of microvascular damage.
5. The combined biomarker model demonstrated **excellent diagnostic accuracy** (AUC 0.90–0.93), supporting its potential clinical utility.

5. Discussion

The study demonstrates that biomarker profiling using HbA1c, fructosamine, and AGEs significantly enhances the early detection of microvascular complications in T2DM. AGEs showed the strongest association with early neuropathy, highlighting the long-term damaging effect of protein glycation. Fructosamine correlated more strongly with early nephropathy, indicating its usefulness in capturing short-term glycemic variation relevant to renal microvasculature.

The combined biomarker model produced superior predictive accuracy, supporting the concept that multi-marker panels outperform individual tests. This aligns with emerging evidence that hyperglycemia-induced pathways are diverse, and no single biomarker fully captures the complexity of metabolic stress in diabetes.

Early identification using biomarker profiling may help clinicians initiate timely interventions such as therapy intensification, lifestyle modification, and targeted screening.

6. Conclusion

Combining HbA1c, fructosamine, and AGEs improves the prediction of early microvascular complications in T2DM. Biomarker profiling could be incorporated into routine diabetes assessments to identify high-risk individuals earlier, potentially preventing long-term disability.

7. Recommendations

- Use profiling for high-risk groups (long duration of diabetes, uncontrolled glycemia).
- Integrate AGEs measurement into tertiary care diabetic clinics.
- Conduct longitudinal studies to validate predictive performance.
- Develop point-of-care AGE detection devices for broader screening.

8. Limitations

- Cross-sectional design limits causal inference.
- AGE measurements are not widely available in routine laboratories.
- Confounding variables (diet, oxidative stress, lipid levels) may affect biomarker concentrations.

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