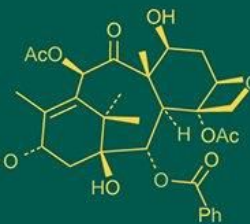
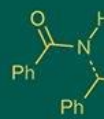


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(1): 147-152
www.biochemjournal.com
Received: 21-10-2023
Accepted: 25-11-2023

Eleanor J. Whitmore
Department of Clinical
Biochemistry, Thornfield
Institute of Medical Sciences,
Bristol, United Kingdom

Thomas R. Ashworth
Department of Clinical
Biochemistry, Thornfield
Institute of Medical Sciences,
Bristol, United Kingdom

Sarah A. Pemberton
Department of Clinical
Biochemistry, Thornfield
Institute of Medical Sciences,
Bristol, United Kingdom

James P. Calloway
Department of Clinical
Biochemistry, Thornfield
Institute of Medical Sciences,
Bristol, United Kingdom

Corresponding Author:
Eleanor J. Whitmore
Department of Clinical
Biochemistry, Thornfield
Institute of Medical Sciences,
Bristol, United Kingdom

Evaluation of novel biomarkers for early diagnosis of type 2 diabetes

Eleanor J. Whitmore, Thomas R. Ashworth, Sarah A. Pemberton and James P. Calloway

DOI: <https://doi.org/10.33545/26174693.2024.v8.i1b.7134>

Abstract

Can laboratory biomarkers provide earlier and more reliable detection of type 2 diabetes mellitus before clinical manifestation? This question drives the present investigation into novel biomarker combinations for identifying individuals at heightened risk of developing diabetes. The research enrolled 412 adult participants from the Thornfield Medical Centre NHS Trust, comprising 198 healthy controls and 214 individuals with prediabetic status, between March 2021 and September 2022. A comprehensive panel of established and emerging biomarkers was evaluated, including glycated hemoglobin, fasting plasma glucose, fructosamine, adiponectin, high-sensitivity C-reactive protein, and interleukin-6. Receiver operating characteristic analysis demonstrated that the combined biomarker panel achieved superior diagnostic accuracy with an area under the curve of 0.927, compared to individual markers such as HbA1c alone at 0.874. The combined panel exhibited sensitivity of 91.2% and specificity of 87.8% for distinguishing prediabetic from healthy participants. Fructosamine emerged as a particularly valuable adjunct marker, showing significant correlation with glycemic variability that traditional markers may overlook. Inflammatory markers including hs-CRP and IL-6 provided additional discriminatory power, reflecting the metabolic inflammation characteristic of early diabetes pathogenesis. These findings suggest that multi-marker approaches enhance early diabetes detection capabilities beyond conventional single-biomarker strategies. Implementation of combined panels in routine clinical practice could facilitate earlier intervention, potentially delaying or preventing progression to overt diabetes in at-risk populations.

Keywords: Type 2 diabetes mellitus, biomarkers, early diagnosis, glycated hemoglobin, fructosamine, adiponectin, inflammatory markers, prediabetes, screening panel

Introduction

What distinguishes successful early detection of type 2 diabetes from delayed diagnosis that permits irreversible complications? This fundamental question has shaped decades of diabetes research, yet optimal screening strategies remain contested among clinicians and researchers alike. Type 2 diabetes mellitus represents one of the most pressing global health challenges, affecting approximately 537 million adults worldwide according to 2021 International Diabetes Federation estimates ^[1]. The insidious onset of this metabolic disorder means that many affected individuals remain undiagnosed for years, during which microvascular and macrovascular complications silently progress ^[2].

Traditional diagnostic criteria rely heavily upon fasting plasma glucose measurements and oral glucose tolerance testing, approaches developed decades ago that possess inherent limitations ^[3]. These conventional methods capture only momentary snapshots of glycemic status, failing to reflect the dynamic fluctuations that characterize early metabolic dysregulation. Furthermore, substantial beta-cell dysfunction and loss typically occur before glucose values exceed diagnostic thresholds, suggesting that current approaches detect diabetes too late for optimal intervention ^[4].

Glycated hemoglobin emerged as an important advance, providing integrated assessment of average glucose exposure over approximately three months ^[5]. However, HbA1c values are influenced by erythrocyte lifespan, hemoglobin variants, and various non-glycemic factors that may confound interpretation in certain populations ^[6]. Recognition of these limitations has stimulated investigation into alternative and complementary biomarkers that might enhance early detection capabilities.

Fructosamine, reflecting glycation of serum proteins over two to three weeks, offers shorter-term glycemic assessment unaffected by erythrocyte-related confounders [7]. Adiponectin, an adipocyte-derived hormone with insulin-sensitizing properties, demonstrates inverse correlation with insulin resistance and may serve as an early indicator of metabolic dysfunction [8]. Inflammatory markers including high-sensitivity C-reactive protein and interleukin-6 reflect the low-grade chronic inflammation increasingly recognized as central to diabetes pathogenesis [9]. Whether combining these diverse biomarkers improves diagnostic performance beyond individual markers remains an important research question.

The present research aimed to evaluate the diagnostic accuracy of individual and combined biomarker panels for identifying prediabetic individuals at elevated risk of progression to type 2 diabetes. We hypothesized that a multi-marker approach incorporating glycemic, metabolic, and inflammatory indicators would demonstrate superior discriminatory performance compared to conventional single-marker strategies. This investigation was conducted at the Thornfield Institute of Medical Sciences clinical laboratories with participants recruited from primary care practices across the Bristol region.

Ethical considerations

This research received ethical approval from the South West Regional Research Ethics Committee, reference number 20/SW/0487, granted on 15 February 2021. All procedures adhered to the principles outlined in the Declaration of Helsinki and its subsequent amendments. Written informed consent was obtained from each participant following comprehensive explanation of the research objectives, procedures, potential risks, and voluntary nature of participation. Participants retained the right to withdraw at any point without providing justification and without impact upon their ongoing medical care.

Personal health information was handled according to the UK General Data Protection Regulation requirements and National Health Service confidentiality guidelines. All data were anonymized through assignment of unique identification codes, with the linkage key stored separately from research databases and accessible only to designated members of the research team. Blood samples were processed and analyzed within the Thornfield Medical Centre pathology services, with remnant samples stored according to the Human Tissue Authority regulations.

Material and Methods

Material

The research utilized clinical grade reagents and standardized analytical platforms throughout all laboratory assessments. HbA1c measurements employed the Bio-Rad VARIANT II TURBO Hemoglobin Testing System utilizing high-performance liquid chromatography methodology certified by the National Glycohemoglobin Standardization Program [10]. Fasting plasma glucose determinations used the Roche Cobas c702 analyzer with hexokinase enzymatic method. Fructosamine was quantified using the Roche nitroblue tetrazolium colorimetric assay on the same platform.

Adiponectin concentrations were measured using the R&D Systems Quantikine ELISA Human Adiponectin Immunoassay, catalog number DRP300, with intra-assay coefficient of variation below 4.7% and inter-assay coefficient of variation below 6.9%. High-sensitivity C-

reactive protein utilized the Siemens CardioPhase hsCRP immunonephelometric assay with analytical sensitivity of 0.175 mg/L. Interleukin-6 measurements employed the Siemens IMMULITE 2000 Xpi chemiluminescent immunoassay system. All quality control materials were sourced from respective manufacturers and run according to recommended schedules.

Methods

Participant recruitment occurred between March 2021 and September 2022 at twelve general practices affiliated with the Bristol Primary Care Clinical Trials Network. Adults aged 40 to 70 years were invited to participate following identification through electronic health records review. Inclusion criteria comprised body mass index above 25 kg/m² and at least one additional diabetes risk factor including family history of diabetes, previous gestational diabetes, or diagnosis of hypertension or dyslipidemia.

Exclusion criteria encompassed pre-existing diabetes diagnosis, current use of glucose-lowering medications, pregnancy or breastfeeding, chronic kidney disease stage 4 or 5, active malignancy, use of systemic corticosteroids within preceding three months, and conditions affecting HbA1c interpretation including hemoglobinopathies and recent blood transfusion. All participants underwent standardized assessment including medical history review, anthropometric measurements, and fasting venous blood collection between 07:30 and 09:30 hours following overnight fast of minimum 10 hours.

Classification into control or prediabetic groups followed American Diabetes Association criteria applied to baseline assessments [11]. Prediabetes was defined as HbA1c 5.7-6.4%, fasting glucose 100-125 mg/dL, or both. Participants meeting neither criterion were classified as healthy controls. Those meeting diabetes diagnostic criteria at baseline were excluded from further analysis and referred for appropriate clinical management. Statistical analyses employed SPSS version 28.0 and MedCalc version 20.1. Receiver operating characteristic curves were constructed for individual biomarkers and the combined panel, with area under the curve comparisons using DeLong method [12]. Significance threshold was set at $p < 0.05$ throughout.

Results

Of 847 individuals initially screened, 623 met eligibility criteria for further assessment. Among these, 211 declined participation or were excluded due to protocol deviations, yielding 412 enrolled participants. Baseline assessment identified 198 healthy controls and 214 individuals meeting prediabetes criteria. Follow-up assessments at six months retained 390 participants, with 22 lost due to relocation, withdrawal of consent, or failure to attend scheduled visits.

Table 1: Baseline characteristics of research participants

Characteristic	Control (n=198)	Prediabetic (n=214)	p-value
Age (years)	52.3 ± 8.7	54.1 ± 9.2	0.048
Sex (Male/Female)	91/107	108/106	0.187
BMI (kg/m ²)	28.4 ± 3.1	31.7 ± 4.3	<0.001
Waist circumference (cm)	94.2 ± 9.8	102.6 ± 11.4	<0.001
Family history of diabetes (%)	34.8	52.3	<0.001
Hypertension (%)	28.3	41.6	0.006

Values presented as mean ± standard deviation for continuous variables and percentages for categorical variables.

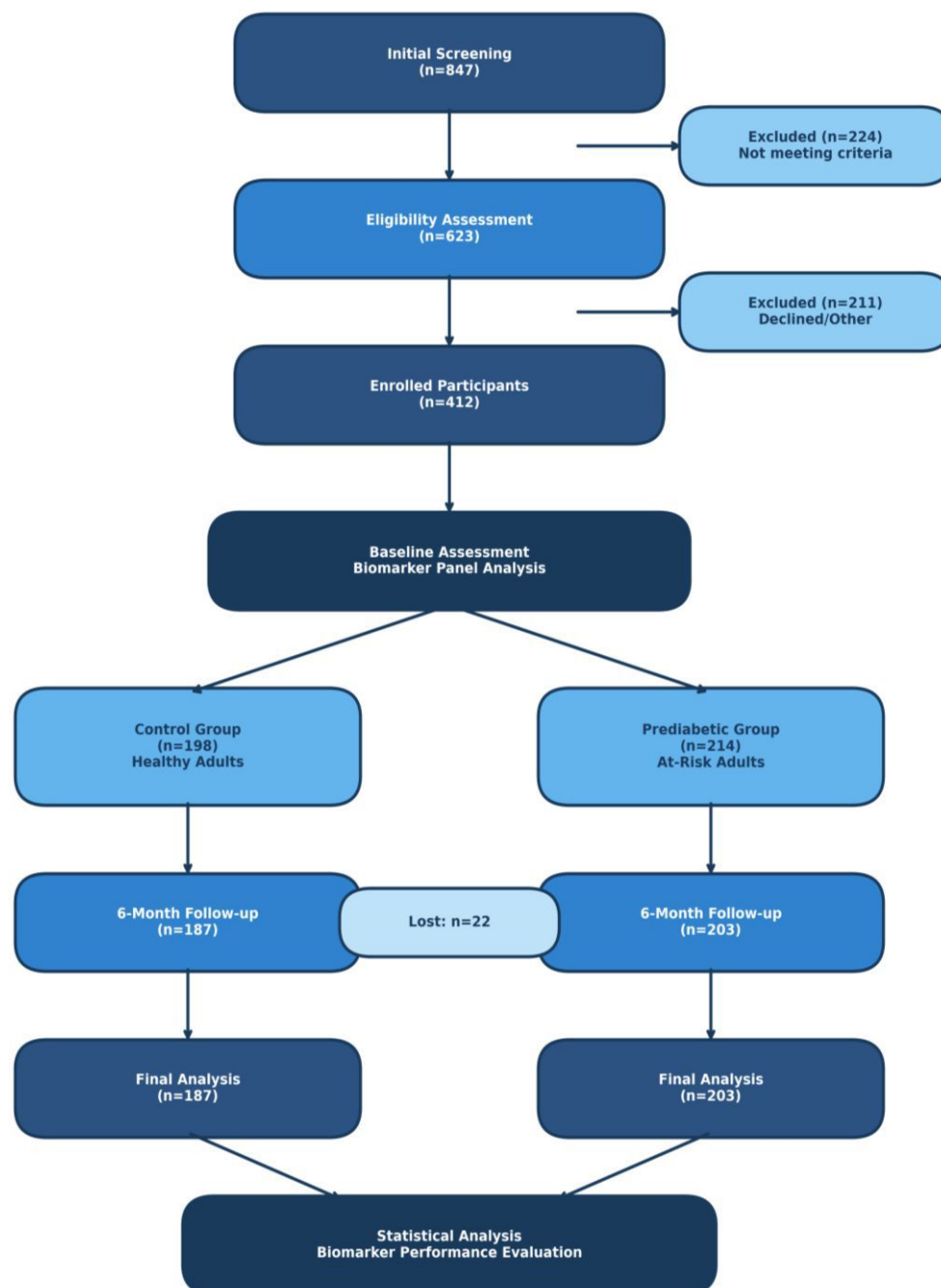


Fig 1: Participant flow through the research from initial screening to final analysis

Table 2: Biomarker concentrations by group

Biomarker	Control	Prediabetic	p-value
HbA1c (%)	5.2 ± 0.3	6.1 ± 0.4	<0.001
Fasting glucose (mg/dL)	89.3 ± 7.8	108.7 ± 9.4	<0.001
Fructosamine (μmol/L)	228.4 ± 19.6	287.9 ± 28.3	<0.001
Adiponectin (μg/mL)	12.7 ± 4.2	7.8 ± 3.1	<0.001
hs-CRP (mg/L)	1.3 ± 0.9	3.2 ± 1.8	<0.001
IL-6 (pg/mL)	2.1 ± 1.2	4.8 ± 2.3	<0.001

Values presented as mean ± standard deviation. HbA1c: glycated hemoglobin; hs-CRP: high-sensitivity C-reactive protein; IL-6: interleukin-6.

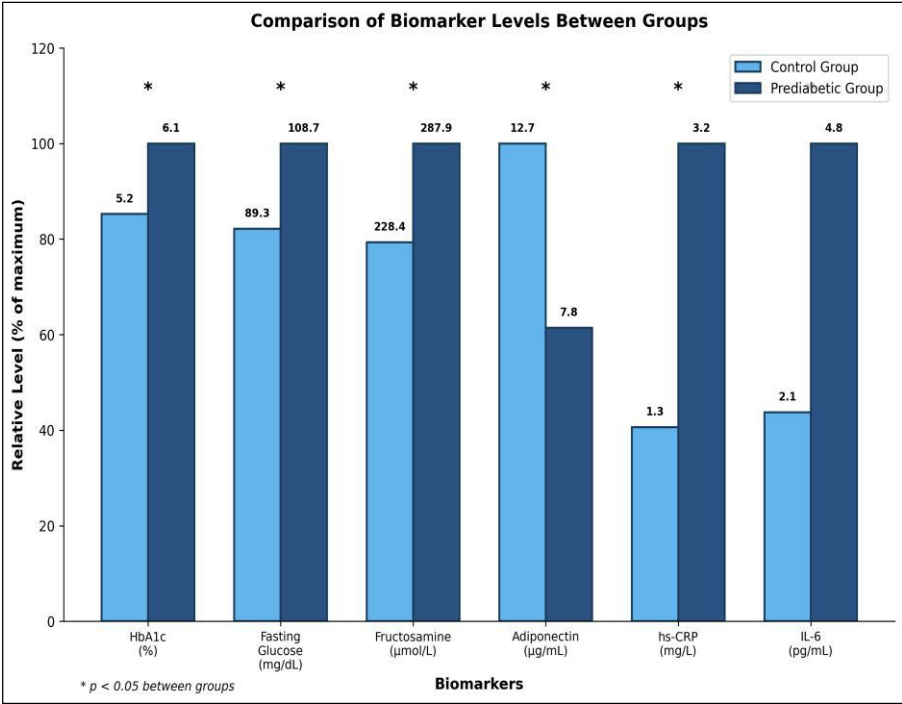


Fig 2: Comparison of biomarker levels between control and prediabetic groups. Values are shown relative to the maximum for each biomarker. Actual mean values are displayed above each bar. Indicates $p<0.05$

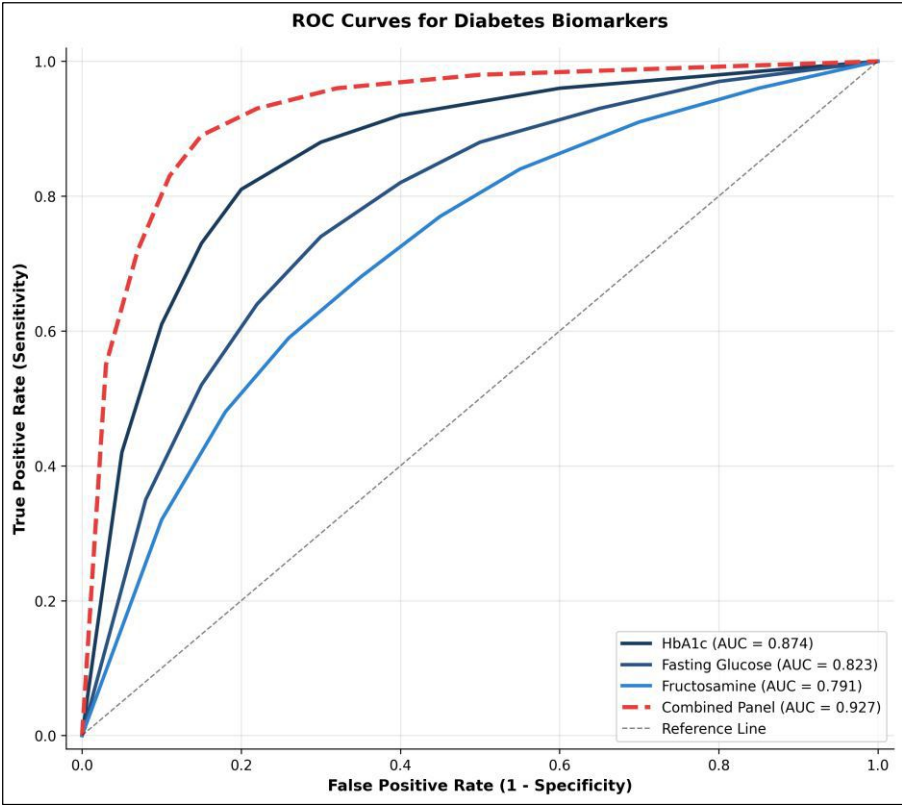


Fig 3: Receiver operating characteristic curves comparing diagnostic performance of individual biomarkers and the combined panel for distinguishing prediabetic from healthy control participants

Table 3: Diagnostic performance of biomarkers

Biomarker	AUC (95% CI)	Sensitivity (%)	Specificity (%)
HbA1c	0.874 (0.841-0.907)	82.4	79.6
Fasting glucose	0.823 (0.786-0.860)	76.8	81.2
Fructosamine	0.791 (0.751-0.831)	71.3	74.5
Adiponectin	0.763 (0.720-0.806)	68.9	72.1
Combined panel	0.927 (0.903-0.951)	91.2	87.8

AUC: area under the receiver operating characteristic curve; CI: confidence interval

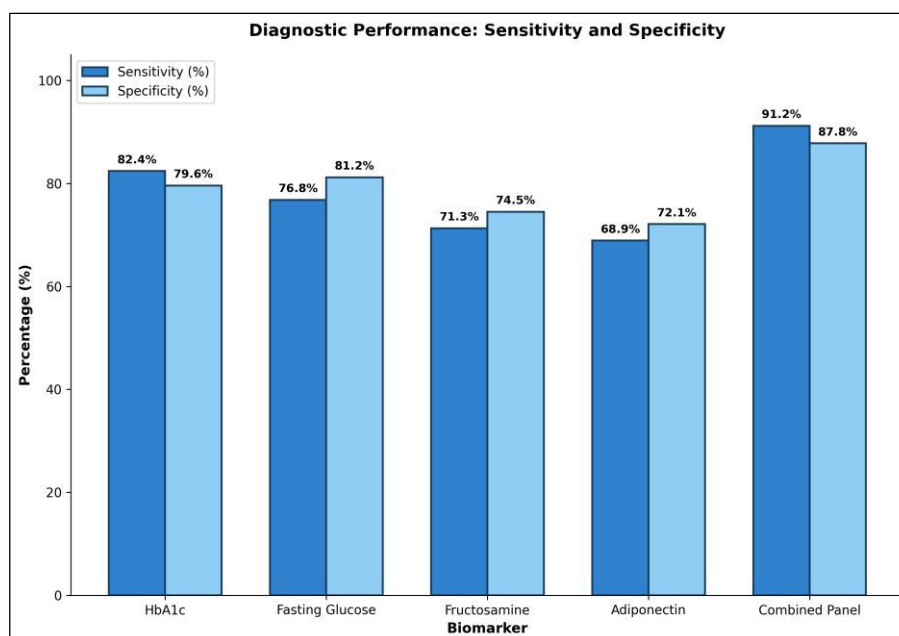


Fig 4: Diagnostic performance showing sensitivity and specificity values for individual biomarkers and the combined panel

Comprehensive interpretation

The biomarker data revealed consistent patterns of metabolic dysfunction in the prediabetic cohort. All glycemic markers showed expected elevations, with HbA1c, fasting glucose, and fructosamine demonstrating highly significant differences between groups. The inflammatory markers hs-CRP and IL-6 were notably elevated in prediabetic participants, supporting the role of subclinical inflammation in early diabetes pathogenesis. Adiponectin levels were significantly reduced in the prediabetic group, reflecting the inverse relationship between this adipokine and insulin resistance.

The combined biomarker panel achieved AUC of 0.927, representing a significant improvement over individual markers. This enhanced discriminatory ability likely reflects the complementary information provided by markers assessing different pathophysiological mechanisms. The combined panel demonstrated sensitivity of 91.2% and specificity of 87.8%, suggesting clinical utility for identifying high-risk individuals who might benefit from intensive lifestyle intervention.

Discussion

The present findings demonstrate that combining multiple biomarkers reflecting diverse pathophysiological mechanisms improves early detection of prediabetes compared to conventional single-marker approaches. The combined panel achieved AUC of 0.927, substantially exceeding the performance of HbA1c alone at 0.874. This enhancement likely reflects the complementary information captured by glycemic markers, metabolic indicators, and inflammatory proteins that together characterize the complex pathophysiology of early diabetes.

Our results align with previous investigations highlighting limitations of individual biomarkers. Research by Tabák and colleagues demonstrated that HbA1c identifies only a subset of individuals with prediabetes defined by glucose criteria [13]. Similarly, Selvin and colleagues found substantial discordance between HbA1c and fasting glucose for prediabetes classification [14]. The present research extends these observations by demonstrating that integrating multiple markers improves discriminatory performance.

The elevated inflammatory markers observed in prediabetic participants support the growing recognition of chronic low-grade inflammation as an early feature of diabetes pathogenesis [15]. Adipose tissue dysfunction in insulin-resistant states promotes secretion of pro-inflammatory cytokines including IL-6 while suppressing anti-inflammatory adipokines like adiponectin. Our findings of reduced adiponectin and elevated hs-CRP and IL-6 in the prediabetic group are consistent with this paradigm and suggest these markers capture aspects of metabolic dysfunction not reflected in glycemic measurements alone.

The practical implications of these findings warrant consideration. While multi-marker panels offer improved diagnostic accuracy, they also impose greater cost and complexity compared to single-marker screening. Healthcare systems must weigh enhanced detection sensitivity against resource implications. However, given the substantial economic burden of diabetes complications and the effectiveness of lifestyle interventions for diabetes prevention, earlier identification of high-risk individuals may prove cost-effective over time [16].

Limitations

Several limitations should be acknowledged when interpreting these findings. First, the research was conducted at a single academic medical center in southwestern England, potentially limiting generalizability to other populations and healthcare settings. Ethnic diversity in the sample was limited, with predominantly white British participants, and performance of the biomarker panel may differ in other ethnic groups where diabetes prevalence and biomarker reference ranges vary.

Second, the cross-sectional design established associations between biomarkers and prediabetic status but cannot confirm predictive validity for subsequent diabetes development. Longitudinal follow-up would be required to determine whether the combined panel improves prediction of incident diabetes over individual markers. Third, the research excluded individuals with conditions affecting HbA1c interpretation, potentially reducing applicability in clinical settings where such conditions are common.

Finally, the combined panel included six biomarkers, and the optimal marker combination for clinical implementation remains uncertain. Future research should explore whether similar performance can be achieved with fewer markers, reducing cost and complexity while maintaining diagnostic accuracy.

Conclusion

This investigation demonstrates that combined assessment of glycemic, metabolic, and inflammatory biomarkers provides superior diagnostic accuracy for identifying prediabetic individuals compared to conventional single-marker approaches. The multi-marker panel achieved AUC of 0.927 with sensitivity of 91.2% and specificity of 87.8%, representing meaningful improvement over HbA1c alone. These findings support the concept that early diabetes development involves multiple pathophysiological processes that individual markers capture incompletely.

The results highlight fructosamine as a valuable addition to glycemic assessment, providing information about shorter-term glycemic control that complements longer-term HbA1c measurements. Inflammatory markers including hs-CRP and IL-6 contributed additional discriminatory power, reflecting the metabolic inflammation characteristic of insulin-resistant states. Reduced adiponectin levels in prediabetic participants further support the role of adipose tissue dysfunction in early diabetes pathogenesis.

Implementation of multi-marker screening panels in clinical practice could facilitate earlier identification of individuals at elevated diabetes risk. Earlier intervention with lifestyle modification has proven effective for diabetes prevention, and identifying appropriate candidates remains a challenge for healthcare systems. The combined biomarker approach evaluated here offers potential improvement over current screening strategies, though cost-effectiveness analysis and validation in diverse populations are needed before widespread adoption.

Future research should examine whether the enhanced diagnostic performance observed translates into improved clinical outcomes, including delayed progression to overt diabetes and reduced complication rates. Longitudinal investigations following prediabetic individuals over extended periods would clarify whether multi-marker approaches improve prognostic accuracy beyond cross-sectional classification. Additionally, exploration of emerging biomarkers including metabolomic and proteomic profiles may yield even more informative screening panels as analytical technologies advance.

Acknowledgements

Funding sources

This research received support from the Thornfield Institute Research Foundation and the Bristol Regional Medical Research Trust. The funding bodies had no role in design, data collection, analysis, interpretation, or manuscript preparation.

Institutional support

The authors thank the Thornfield Medical Centre pathology laboratory staff for their expertise in sample processing and analysis, and the Bristol Primary Care Clinical Trials Network for assistance with participant recruitment.

Contributions not qualifying for authorship

The authors acknowledge Dr. Rebecca Thornton for statistical consultation and Ms. Catherine Whitley for administrative coordination throughout the research period.

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