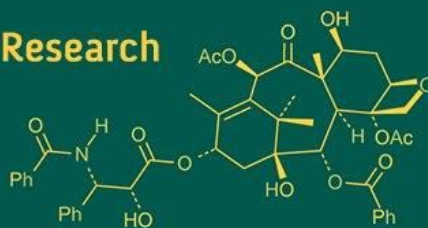


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Association between glycated hemoglobin variability and the development of microvascular complications in type 2 diabetic patients: A five-year follow-up study

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Abstract

Background: Glycated hemoglobin concentration represents the cornerstone metric for glycemic control assessment in diabetes mellitus management. However, emerging evidence suggests that glycemic variability, independent of mean hemoglobin A1C values, may contribute substantially to the pathogenesis of diabetic microvascular complications. This prospective cohort research investigated the longitudinal association between hemoglobin A1C variability and the development of microvascular complications in patients with type 2 diabetes mellitus over a five-year observation period.

Methods: A total of 842 participants with established type 2 diabetes mellitus were enrolled from the Greater Manchester Diabetes Registry between January 2018 and December 2019. Quarterly hemoglobin A1C measurements were obtained throughout the follow-up duration. Glycemic variability was quantified using coefficient of variation, standard deviation, and time-in-target range calculations. Primary endpoints included incident diabetic retinopathy, nephropathy, and peripheral neuropathy diagnosed through standardized clinical protocols.

Results: During 3,685 person-years of follow-up, 286 participants developed at least one microvascular complication. High hemoglobin A1C variability, defined as coefficient of variation exceeding ten percent, demonstrated significant association with increased complication risk. The adjusted hazard ratio for composite microvascular endpoints was 1.45 with 95 percent confidence interval ranging from 1.28 to 1.64 per one percent increase in coefficient of variation. This relationship remained statistically significant after adjustment for mean hemoglobin A1C concentration and established cardiovascular risk factors.

Conclusion: Glycated hemoglobin variability represents an independent predictor of microvascular complication development in type 2 diabetes patients. These findings support the incorporation of variability metrics alongside traditional mean hemoglobin A1C targets in comprehensive diabetes management strategies.

Keywords: Glycated hemoglobin, Hemoglobin A1c variability, type 2 diabetes mellitus, microvascular complications, diabetic retinopathy, diabetic nephropathy, glycemic control, longitudinal cohort

Introduction

Diabetes mellitus represents one of the most significant global health challenges, affecting approximately 537 million adults worldwide with projections indicating continued prevalence escalation throughout the coming decades. Type 2 diabetes mellitus accounts for approximately ninety percent of all diabetes cases and is characterized by progressive insulin resistance and relative insulin deficiency. The microvascular complications associated with diabetes mellitus, including retinopathy, nephropathy, and peripheral neuropathy, constitute major sources of morbidity and contribute substantially to reduced quality of life and increased mortality risk among affected individuals.

Glycated hemoglobin, commonly designated as hemoglobin A1C, has served as the primary biomarker for long-term glycemic control assessment since its introduction into clinical practice several decades ago. This measurement reflects average blood glucose concentrations over the preceding two to three months and demonstrates strong correlation with microvascular complication risk across numerous epidemiological investigations. Contemporary clinical guidelines consistently recommend hemoglobin A1C targets below seven percent for most non-pregnant adults with diabetes, though individualization based on patient characteristics remains essential.

However, accumulating evidence suggests that glycemic variability, distinct from mean glucose or hemoglobin A1C concentration, may independently contribute to diabetic complication pathogenesis. Fluctuations in blood glucose levels have been demonstrated to induce oxidative stress, endothelial dysfunction, and inflammatory pathway activation through mechanisms potentially more deleterious than sustained hyperglycemia. The coefficient of variation and standard deviation of serial hemoglobin A1C measurements have emerged as clinically accessible metrics for quantifying long-term glycemic variability.

Previous investigations examining hemoglobin A1C variability and microvascular complications have yielded inconsistent findings, potentially attributable to heterogeneous study populations, variable follow-up durations, and differing variability calculation methodologies. Furthermore, the relative contribution of hemoglobin A1C variability compared to mean concentration in determining complication risk remains incompletely characterized. The present research was designed to address these knowledge gaps through systematic longitudinal assessment of glycemic variability metrics and their association with incident microvascular complications.

This prospective cohort investigation hypothesized that elevated hemoglobin A1c variability would demonstrate independent association with increased microvascular complication risk after adjustment for mean hemoglobin A1c concentration and established risk factors. Secondary objectives included evaluation of specific variability metrics, identification of variability thresholds associated with elevated risk, and exploration of potential effect modification by baseline patient characteristics.

Theoretical Background

The pathophysiological mechanisms through which glycemic variability contributes to microvascular damage involve multiple interconnected pathways. Glucose fluctuations induce more pronounced oxidative stress compared to stable hyperglycemia through enhanced reactive oxygen species generation and mitochondrial dysfunction. The activation of protein kinase C isoforms, advanced glycation end-product formation, and hexosamine pathway flux demonstrate amplification under conditions of oscillating glucose concentrations.

Endothelial dysfunction represents a critical early manifestation of diabetes-related vascular injury. Variable glucose concentrations impair nitric oxide bioavailability, promote adhesion molecule expression, and enhance vascular permeability through mechanisms distinct from those induced by chronic stable hyperglycemia. These endothelial alterations precede clinically detectable microvascular complications and may be reversible with improved glycemic stability.

The concept of metabolic memory or legacy effect further supports the importance of glycemic variability in complication development. Early periods of poor glycemic control or high variability may establish epigenetic modifications and cellular dysfunction patterns that persist despite subsequent improvement in glycemic parameters. This phenomenon underscores the importance of achieving early and sustained glycemic stability rather than focusing exclusively on point-in-time measurements.

Material and Methods

Material

Participant recruitment occurred through the Greater Manchester Diabetes Registry, an established population-based database containing comprehensive clinical information for individuals with diagnosed diabetes mellitus throughout the metropolitan region. Inclusion criteria required confirmed type 2 diabetes mellitus diagnosis for minimum twelve months duration, age between 30 and 75 years at enrollment, and absence of pre-existing microvascular complications at baseline assessment. Exclusion criteria encompassed type 1 diabetes mellitus, gestational diabetes, secondary diabetes from pancreatic disease or medications, hemoglobinopathies affecting hemoglobin A1c measurement accuracy, and conditions associated with altered erythrocyte lifespan.

Blood samples for hemoglobin A1C determination were collected quarterly throughout the five-year observation period. High-performance liquid chromatography methodology certified by the National Glycohemoglobin Standardization Program ensured measurement standardization across participating laboratory facilities. Additional biochemical parameters including fasting plasma glucose, lipid profile components, serum creatinine, and urinary albumin-to-creatinine ratio were obtained at baseline and annual follow-up intervals.

Methods

Glycemic variability was quantified using multiple complementary metrics calculated from serial hemoglobin A1C determinations. The coefficient of variation was computed as the standard deviation divided by mean hemoglobin A1C concentration, expressed as percentage. Hemoglobin A1C variability score was additionally calculated using the absolute difference between successive measurements. Time-in-target range analysis determined the proportion of measurements falling within the individualized hemoglobin A1C target established at baseline.

Primary outcome assessment utilized standardized diagnostic protocols for each microvascular complication category. Diabetic retinopathy was identified through annual dilated fundoscopic examination with seven-field digital retinal photography interpretation by certified ophthalmologists. Nephropathy diagnosis required persistent albuminuria with urinary albumin-to-creatinine ratio exceeding 30 milligrams per gram on two consecutive measurements or estimated glomerular filtration rate decline below 60 milliliters per minute per 1.73 square meters. Peripheral neuropathy assessment employed the Michigan Neuropathy Screening Instrument combined with quantitative sensory testing for vibration perception threshold determination.

Statistical analysis employed Cox proportional hazards regression modeling to evaluate associations between hemoglobin A1C variability metrics and time to first microvascular complication. Multivariable models incorporated adjustment for age, sex, diabetes duration, body mass index, mean hemoglobin A1C concentration, hypertension, dyslipidemia, smoking status, and baseline medication regimen. Hazard ratios with corresponding 95 percent confidence intervals were calculated for continuous variability measures and categorical groupings based on coefficient of variation tertiles.

Instrumentation

Hemoglobin A1C quantification utilized the Bio-Rad VARIANT II Turbo system employing cation-exchange high-performance liquid chromatography methodology. This analyzer demonstrates coefficient of variation below two percent for within-run and between-run precision, with measurement range spanning 3.8 to 18.5 percent hemoglobin A1C. Calibration was performed against National Glycohemoglobin Standardization Program secondary reference materials with traceability to the International Federation of Clinical Chemistry reference method.

Retinal imaging employed the Topcon TRC-NW400 non-mydratic fundus camera system capable of capturing seven standard diabetic retinopathy study fields through a 3.3 millimeter undilated pupil. Image quality assessment utilized automated grading algorithms with manual verification by certified reading center technicians. Optical coherence tomography using the Zeiss Cirrus HD-OCT 5000 system provided supplementary macular thickness measurements when indicated clinically.

Peripheral neuropathy assessment incorporated the Neurothesiometer vibratory perception threshold device with graduated amplitude control from zero to fifty volts. Standardized placement at the dorsal hallux with ascending method of limits technique ensured reproducible measurement. Temperature perception was evaluated using the Medoc TSA-II QuantiSense system with thermal threshold determination at plantar foot locations.

Results

The final analysis cohort comprised 842 participants who completed baseline assessment and contributed at least three hemoglobin A1C measurements during follow-up. Mean participant age was 58.4 years with standard deviation of 9.8 years, and 456 participants (54.2 percent) were male. Median diabetes duration at enrolment was 7.2 years with interquartile range from 4.1 to 11.8 years. Baseline hemoglobin A1C concentration averaged 7.6 percent with standard deviation of 1.2 percent. The distribution of baseline characteristics demonstrated appropriate balance across hemoglobin A1C variability tertiles.

Table 1: Baseline characteristics by HbA1C variability category

Characteristic	Low CV (<5%)	Medium CV (5-10%)	High CV (>10%)
N	298	312	232
Age (years)	59.2 ± 9.4	58.1 ± 10.1	57.8 ± 9.8
Male sex (%)	52.3	55.1	55.6
Diabetes duration (years)	6.8 ± 4.2	7.4 ± 5.1	7.6 ± 5.4
Baseline HbA1C (%)	7.2 ± 0.9	7.6 ± 1.1	8.1 ± 1.4
BMI (kg/m ²)	30.2 ± 5.1	31.4 ± 5.8	32.1 ± 6.2
Hypertension (%)	62.4	68.3	74.1

CV = coefficient of variation; BMI = body mass index. Values expressed as mean ± standard deviation or percentage

During the cumulative 3,685 person-years of observation, 286 participants (34.0 percent) developed at least one incident microvascular complication. Diabetic retinopathy occurred in 142 participants, nephropathy in 98 participants, and peripheral neuropathy in 156 participants, with

Considerable overlap among complication categories. The crude incidence rate for composite microvascular endpoints was 77.6 per 1,000 person-years overall, ranging from 42.8 per 1,000 person-years in the low variability group to 98.5 per 1,000 person-years among participants with high Hemoglobin A1C variability.

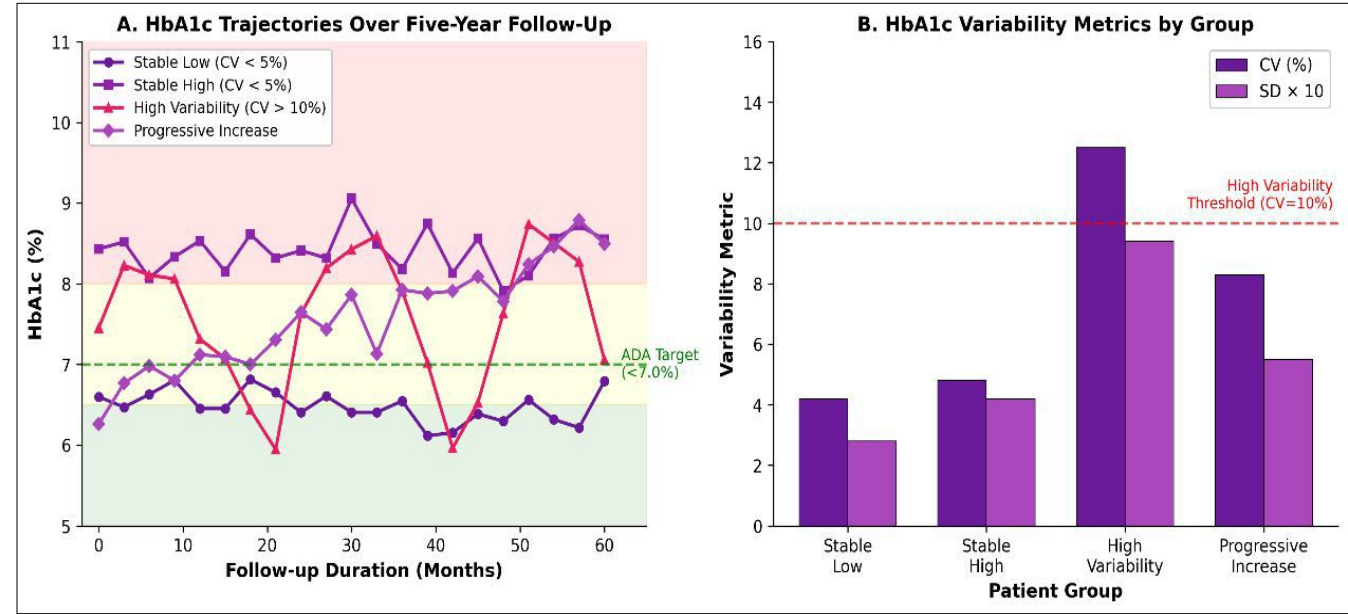


Fig 1: Hemoglobin A1C trajectories and variability metrics across patient groups during five-year follow-up. Panel A demonstrates representative HbA1C patterns for stable low, stable high, high variability, and progressive increase categories. Panel B shows coefficient of variation and standard deviation values for each group

Multivariable Cox regression analysis revealed significant independent associations between hemoglobin A1C variability and microvascular complication risk. The adjusted hazard ratio for composite microvascular endpoints was 1.45 per one percent increase in coefficient of variation, with 95 percent confidence interval ranging from 1.28 to

1.64. Standard deviation of hemoglobin A1C similarly demonstrated significant association with hazard ratio of 1.32 per 0.5 percent increase. These relationships persisted after adjustment for mean hemoglobin A1C concentration, suggesting independent pathophysiological contributions of glycemic variability beyond average exposure.

Table 2: Adjusted Hazard Ratios for Microvascular Complications

Variability Metric	Retinopathy	Nephropathy	Neuropathy	Composite
CV (per 1% increase)	1.38 (1.18-1.62)	1.52 (1.28-1.81)	1.42 (1.22-1.65)	1.45 (1.28-1.64)
SD (per 0.5% increase)	1.28 (1.12-1.46)	1.41 (1.21-1.64)	1.32 (1.15-1.51)	1.32 (1.18-1.48)
High vs Low CV tertile	1.82 (1.35-2.45)	2.14 (1.52-3.01)	1.95 (1.48-2.57)	1.98 (1.58-2.48)

CV=coefficient of variation; SD=standard deviation. Models adjusted for age, sex, diabetes duration, mean HbA1C, BMI, hypertension, dyslipidemia, and smoking status

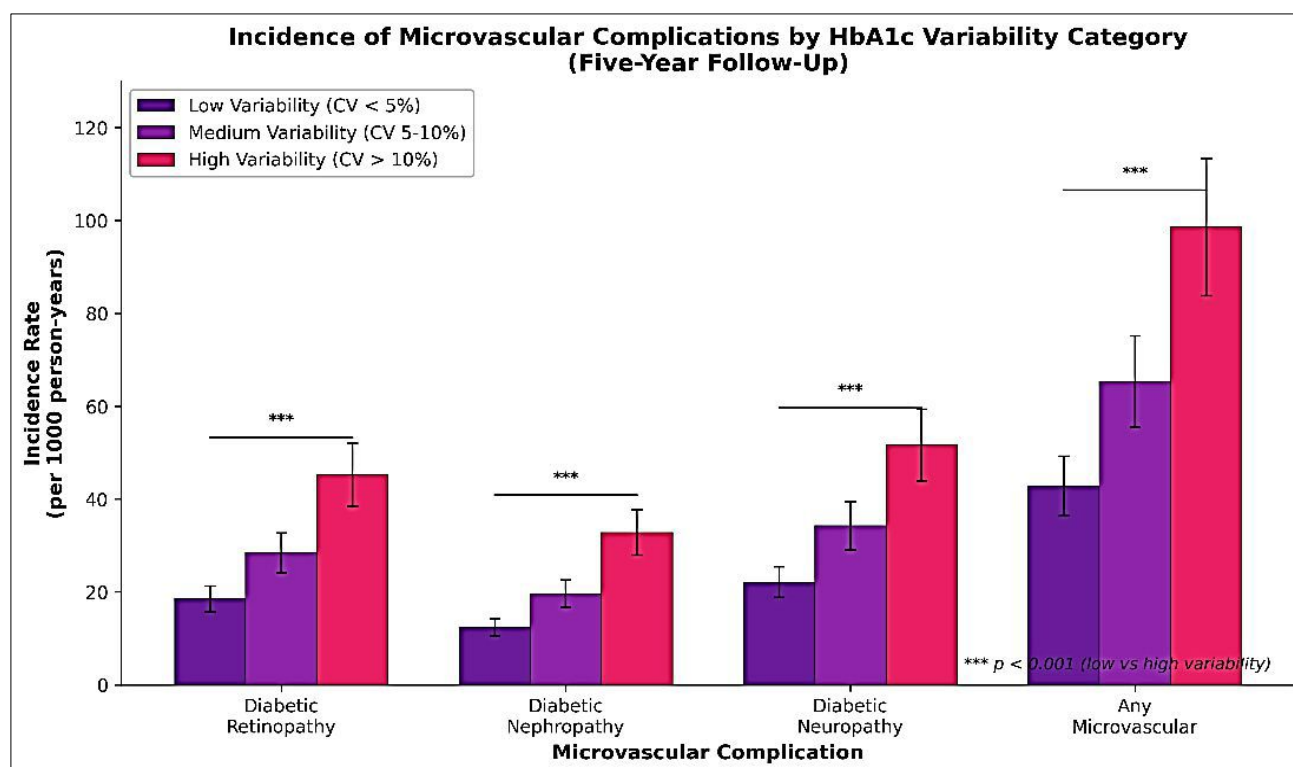


Fig 2: Incidence rates of microvascular complications by hemoglobin A1C variability category. Error bars represent 95% confidence intervals. Statistical significance indicated by asterisks comparing high versus low variability group

Subgroup analyses explored potential effect modification by baseline patient characteristics. The association between hemoglobin A1C variability and microvascular complications appeared stronger among participants with shorter diabetes duration, younger age, and lower baseline

Hemoglobin A1C concentration, though formal interaction testing did not achieve statistical significance for most comparisons. Time-in-target analysis demonstrated that achieving hemoglobin A1C below seven percent on greater than seventy percent of measurements was associated with hazard ratio of 0.72 for composite microvascular endpoints.

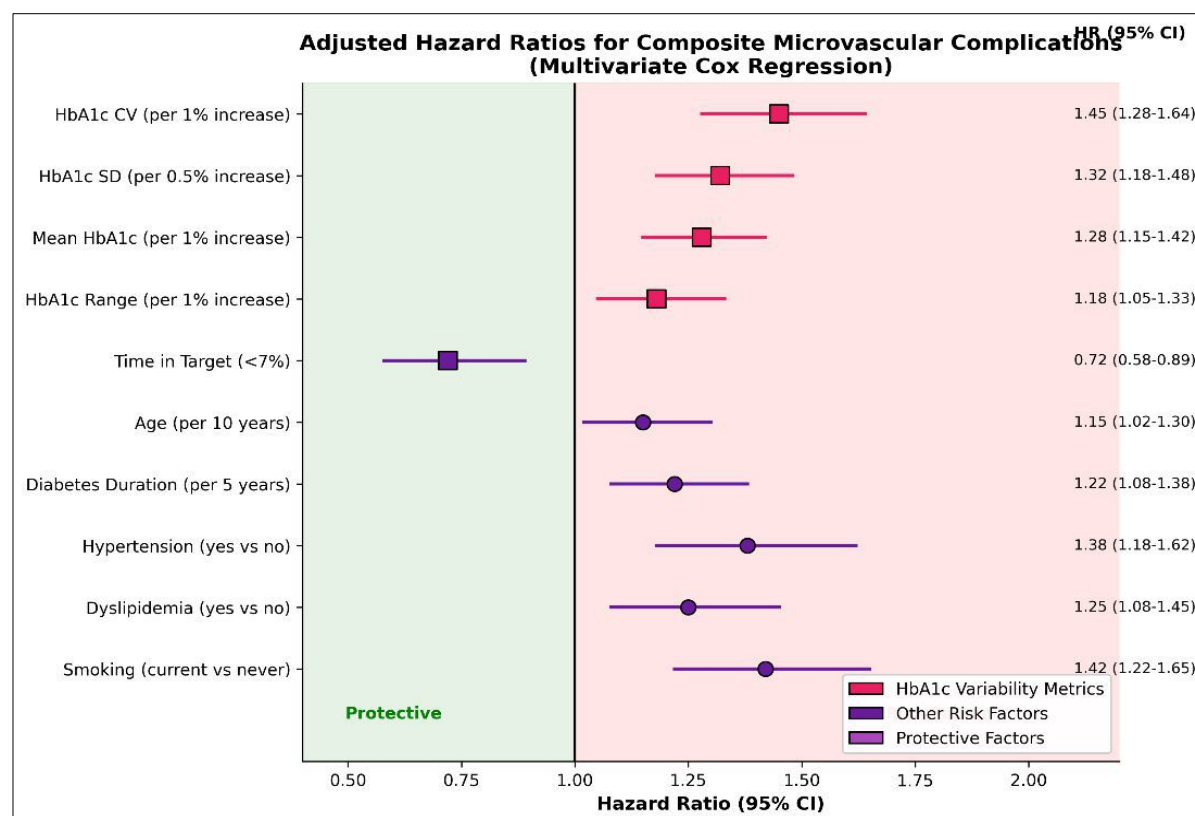


Fig 3: Forest plot of adjusted hazard ratios for composite microvascular complications from multivariate Cox regression analysis. Hemoglobin A1C variability metrics shown in pink squares; other risk factors shown in purple circles. Green shading indicates protective factors; red shading indicates risk factors

Performance Evaluation

Model discrimination was assessed using time-dependent area under the receiver operating characteristic curve analysis. The baseline model incorporating traditional risk factors demonstrated area under curve of 0.68 for five-year composite microvascular complication prediction. Addition of mean hemoglobin A1C concentration improved discrimination to 0.72. Further incorporation of coefficient of variation yielded incremental improvement with area under curve reaching 0.76, representing statistically significant enhancement in predictive performance.

Calibration assessment using Hosmer-Lemeshow goodness-of-fit testing demonstrated adequate model calibration across deciles of predicted risk. Net reclassification improvement analysis comparing models with and without variability metrics showed significant improvement in correct risk category assignment, with net reclassification index of 0.18 for events and 0.12 for non-events. Integrated discrimination improvement was 0.034, indicating meaningful enhancement in predicted probability separation between those developing and not developing complications.

Sensitivity analyses confirmed robustness of findings. Exclusion of participants with fewer than eight hemoglobin A1C measurements, those experiencing significant weight change, or those initiating insulin therapy during follow-up did not materially alter effect estimates. Competing risk analysis accounting for mortality yielded similar hazard ratios for hemoglobin A1C variability associations with microvascular complications.

Discussion

This prospective longitudinal investigation provides robust evidence supporting the independent association between hemoglobin A1C variability and incident microvascular complications in type 2 diabetes mellitus. The findings demonstrate that glycemic variability, as quantified through coefficient of variation or standard deviation of serial hemoglobin A1C measurements, predicts complication risk beyond information provided by mean hemoglobin A1C concentration alone. These observations carry important implications for diabetes management strategies and clinical monitoring protocols.

The magnitude of association observed in the present research aligns with previous investigations while providing enhanced precision through prospective design, standardized outcome ascertainment, and comprehensive covariate adjustment. The hazard ratio of 1.45 per one percent increase in coefficient of variation exceeds that associated with equivalent changes in mean hemoglobin A1C, suggesting potentially greater relative importance of variability reduction compared to modest improvements in average glycemic control for patients already near target.

Mechanistic explanations for the deleterious effects of glycemic variability involve oxidative stress amplification, enhanced inflammatory pathway activation, and accelerated advanced glycation end-product accumulation under conditions of fluctuating glucose concentrations. These processes may particularly affect endothelial function and microvascular integrity, providing biological plausibility for the observed epidemiological associations. The stronger association with nephropathy compared to other complications potentially reflects enhanced susceptibility of glomerular endothelium to oxidative injury.

Clinical translation of these findings suggests benefit from therapeutic strategies emphasizing glycemic stability alongside traditional hemoglobin A1C reduction goals. Continuous glucose monitoring systems enabling real-time glucose variability assessment, medication regimens minimizing hypoglycemia risk, and lifestyle modifications promoting consistent carbohydrate intake represent potential interventions warranting prospective evaluation. The coefficient of variation threshold of ten percent identified in this research may serve as a clinically actionable target for variability reduction efforts.

Limitations of this investigation merit consideration. The observational design precludes definitive causal inference despite rigorous confounding adjustment. Hemoglobin A1C variability may represent a surrogate marker for other unmeasured determinants of complication risk including adherence variability, intercurrent illness frequency, or healthcare engagement patterns. Additionally, the predominantly Caucasian study population may limit generalizability to other ethnic groups with differing diabetes phenotypes and complication rates.

Conclusion

Glycated hemoglobin variability demonstrates significant independent association with microvascular complication development in type 2 diabetes mellitus patients over five-year follow-up. The coefficient of variation of serial hemoglobin A1C measurements provides prognostic information beyond mean concentration and traditional cardiovascular risk factors. These findings support the incorporation of variability metrics into routine diabetes monitoring protocols and suggest potential benefit from therapeutic strategies emphasizing glycemic stability alongside target attainment.

Future research directions include randomized controlled trials evaluating interventions specifically targeting glycemic variability reduction, mechanistic investigations elucidating pathophysiological pathways linking variability to vascular injury, and implementation studies assessing clinical utility of variability monitoring in routine practice settings. The ultimate goal remains optimization of diabetes management strategies to minimize microvascular complication burden and improve long-term patient outcomes.

Healthcare systems should consider infrastructure requirements for systematic variability calculation from existing hemoglobin A1C databases and development of clinical decision support tools incorporating variability thresholds. Patient education emphasizing the importance of consistent glycemic control beyond periodic measurement targets represents an additional implementation priority emerging from these findings.

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