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The Role Of Circulating Microbial DNA (Cf-Mbdna) As An Early Biomarker Of Chronic Inflammation In Long-Standing Diabetes Mellitus And Its Association With Microvascular Complications: A Systematic Review

Abdullah Talal Salem[1], Faisal Abdullah Suwailem Aljohani[2], Nuha Al Yousfi[3], Bashayr Abdullah Alkuhayli[4], Ahmed Shawqi Ebrahim Isa[5], Arwa Waleed Hammouda[6], Yara Mohammed Alamri[7], Abdullah Almohamad Almustafa[8], Alaa Saadallah Habashi[9], Siraj Bandr Fakiha[10], Rahaf Bin Abdullah Abdulaziz Alqahtani[11], Mohammed Abdulghani Alsaeed[12]

[1]Internal Medicine Senior Registrar
[2]Emergency Medicine General Practitioner
[3]Department of Internal Medicine, Faculty of Medicine and Health Sciences, Sana'a University Yemen
[4]Clinical Pharmacist
[5]Medical Intern at SMC (Medical Graduate)
[6]General practitioner
[7]Internal Medicine Senior Resident
[8]Bachelor of Medicine, Bachelor of Surgery
[9]GP, Internal Medicine
[10]Medical Student
[11]Intern
[12]Medical Intern

Abstract

Background: Circulating microbial DNA (cf-mbDNA) and related cell-free DNA (cfDNA) fragments have emerged as novel biomarkers reflecting metabolic stress, inflammation, and vascular injury in diabetes mellitus (DM). This systematic review aimed to synthesize current evidence on their diagnostic potential for detecting chronic inflammation and microvascular complications in long-standing diabetes.
Methods: Following PRISMA 2020 guidelines, ten peer-reviewed studies published between 2010 and 2025 were systematically analyzed across multiple databases. Eligible studies assessed cfDNA or cf-mbDNA in plasma or serum samples from patients with type 1 or type 2 diabetes, investigating associations with inflammatory and vascular markers.
Results: Across the reviewed literature, cfDNA profiles—such as 5-hydroxymethylcytosine patterns, methylation signatures, and microbial fragments—were consistently associated with endothelial dysfunction, renal impairment, and systemic inflammation. Quantitative models based on cfDNA achieved high diagnostic performance, with reported AUC values exceeding 0.85 for identifying vascular complications. Studies further demonstrated that increased cf-mbDNA and decreased mitochondrial cfDNA were linked to heightened inflammatory activity and metabolic dysfunction.
Conclusion: Circulating microbial and cell-free DNA biomarkers represent promising noninvasive indicators of chronic inflammation and early microvascular damage in diabetes. They provide mechanistic insight into the interplay between metabolic dysregulation, immune activation, and vascular injury. Standardized detection methods and longitudinal validation are needed to support their integration into clinical practice.

Keywords: circulating microbial DNA, cfDNA, diabetes mellitus, inflammation, microvascular complications, biomarkers, mitochondrial DNA, epigenetics, nephropathy, retinopathy.

Introduction

Diabetes mellitus (DM) is a multifactorial metabolic disorder characterized by chronic hyperglycemia resulting from insulin resistance, impaired insulin secretion, or both. The long-term persistence of elevated glucose levels triggers widespread oxidative stress and inflammatory cascades, leading to cellular injury and endothelial dysfunction. Over time, these processes culminate in microvascular and macrovascular complications—such as retinopathy, nephropathy, neuropathy, and cardiovascular disease—that significantly contribute to morbidity and mortality among diabetic populations. Despite advances in glycemic monitoring and treatment, early detection of subclinical vascular changes remains a major challenge in diabetes management (Tremblay & Hamet, 2015; Horton & Barrett, 2021).

Chronic inflammation represents a fundamental mechanism driving the progression from metabolic dysregulation to tissue damage in diabetes. Proinflammatory cytokines, including IL-6, TNF-α, and CRP, promote endothelial activation, reduce nitric oxide bioavailability, and impair capillary perfusion. These processes collectively disrupt vascular homeostasis and contribute to retinopathy, nephropathy, and neuropathy. Microvascular dysfunction in diabetes has been documented as both a consequence and amplifier of systemic metabolic stress, forming a bidirectional relationship with insulin resistance and endothelial injury (Horton & Barrett, 2021; Wołoszyn-Durkiewicz & Myśliwiec, 2019).

The discovery of circulating cell-free DNA (cfDNA) in human plasma has transformed biomarker research. cfDNA fragments are released from apoptotic and necrotic cells into the bloodstream, where they reflect dynamic cellular turnover and tissue damage. Advances in molecular techniques, including quantitative PCR and next-generation sequencing, have enabled sensitive detection of cfDNA and its epigenetic modifications. In metabolic diseases, altered cfDNA concentrations have been associated with endothelial dysfunction, inflammation, and oxidative stress, suggesting that cfDNA serves as a non-invasive indicator of diabetic tissue injury (Pollastri, Kovacs, & Keller, 2025; Ranucci, 2018).

Beyond human-derived cfDNA, circulating microbial DNA (cf-mbDNA) has emerged as a novel biomarker of host–microbe interactions. cf-mbDNA originates from commensal and pathogenic microorganisms that translocate across mucosal or endothelial barriers into the bloodstream. Its composition and abundance are influenced by gut permeability, immune activation, and metabolic homeostasis. Recent metagenomic and contaminant-controlled sequencing studies have confirmed that cf-mbDNA can be reliably detected in peripheral blood and carries taxonomic signatures distinct from fecal microbiota (Zozaya-Valdés et al., 2021; Zhai et al., 2024; Pietrzak et al., 2023).

Translocation of microbial products and nucleic acids into circulation contributes to chronic systemic inflammation—a central feature of diabetes. cf-mbDNA can act as a pathogen-associated molecular pattern (PAMP), activating Toll-like receptors and downstream inflammatory cascades. This leads to cytokine release, endothelial injury, and disruption of vascular integrity. In diabetics, elevated cf-mbDNA levels may therefore reflect both microbial dysbiosis and low-grade endotoxemia, perpetuating vascular inflammation and insulin resistance (Pietrzak et al., 2023; Park et al., 2023).

Emerging evidence links cfDNA levels and methylation status to diabetic microvascular complications such as retinopathy and nephropathy. cfDNA derived from damaged endothelial and retinal cells can be detected in plasma before the clinical onset of these complications. For example, hyperglycemia-induced DNA damage and hypomethylation patterns in cfDNA are being explored as early diagnostic signals of diabetic retinopathy (Li et al., 2025). Similarly, increased cfDNA concentrations correlate with albuminuria and glomerular injury, positioning cfDNA as a surrogate marker for renal microvascular stress (El Tarhouny et al., 2010).

Recent studies emphasize that integrating cfDNA methylation signatures with microbial DNA profiling could enhance diagnostic precision. The combination of host-derived epigenetic markers and microbial cfDNA composition provides a multidimensional view of diabetes

pathophysiology, capturing both inflammatory and dysbiotic components. Such integrated biomarkers hold promise for early detection of complications and for evaluating therapeutic responses to anti-inflammatory or metabolic interventions (Li et al., 2025; Pollastri et al., 2025; Zhai et al., 2024).

Given the established role of inflammation and endothelial injury in chronic diabetes, investigating circulating microbial DNA offers a compelling new diagnostic avenue. cf-mbDNA may serve as a real-time, non-invasive biomarker reflecting both metabolic dysregulation and microbial translocation. Its association with microvascular complications could illuminate novel pathways linking gut microbiota, systemic inflammation, and vascular health. Understanding these interconnections is critical for developing predictive models and early-stage interventions to mitigate long-term diabetic morbidity (Pichu et al., 2017; Tremblay & Hamet, 2015; Horton & Barrett, 2021).

Methodology

Study Design

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines to ensure methodological rigor, transparency, and reproducibility. The primary objective was to synthesize and critically appraise empirical evidence examining the role of circulating microbial DNA (cf-mbDNA) and related cell-free DNA (cfDNA) biomarkers as early indicators of chronic inflammation and microvascular complications in long-standing diabetes mellitus (DM).

The review focused on peer-reviewed studies investigating cf-mbDNA, cf-mtDNA, or cf-nDNA as diagnostic or prognostic biomarkers for microvascular complications (e.g., diabetic nephropathy, retinopathy, neuropathy, and cardiovascular dysfunction) in both type 1 and type 2 diabetes. Quantitative, qualitative, and mixed-methods studies were considered to ensure a comprehensive understanding of molecular, microbial, and clinical associations.

Eligibility Criteria

Inclusion Criteria:

- **Population:** Adults diagnosed with type 1 or type 2 diabetes mellitus, with or without microvascular or macrovascular complications.
- **Biomarker Focus:** Studies measuring circulating microbial DNA (cf-mbDNA), cell-free nuclear DNA (cf-nDNA), or cell-free mitochondrial DNA (cf-mtDNA) in plasma or serum.
- **Outcomes:** Associations between cfDNA/cf-mbDNA levels and markers of inflammation (IL-6, TNF-α, CRP), endothelial dysfunction, renal function (eGFR, albuminuria), or vascular complications.
- **Study Designs:** Cross-sectional, case–control, cohort, or interventional studies providing quantitative or molecular data.
- **Language:** English-language publications only.
- **Publication Period:** Studies published between 2010 and 2025, aligning with the period of expanding cfDNA biomarker research.

Exclusion Criteria:

- Reviews, commentaries, conference abstracts, and case reports.
- Studies not involving human participants (e.g., animal or in vitro models).
- Research not reporting cfDNA or cf-mbDNA biomarkers.
- Duplicate publications or studies lacking full-text availability.

Following full-text screening, ten studies met all inclusion criteria for analysis.

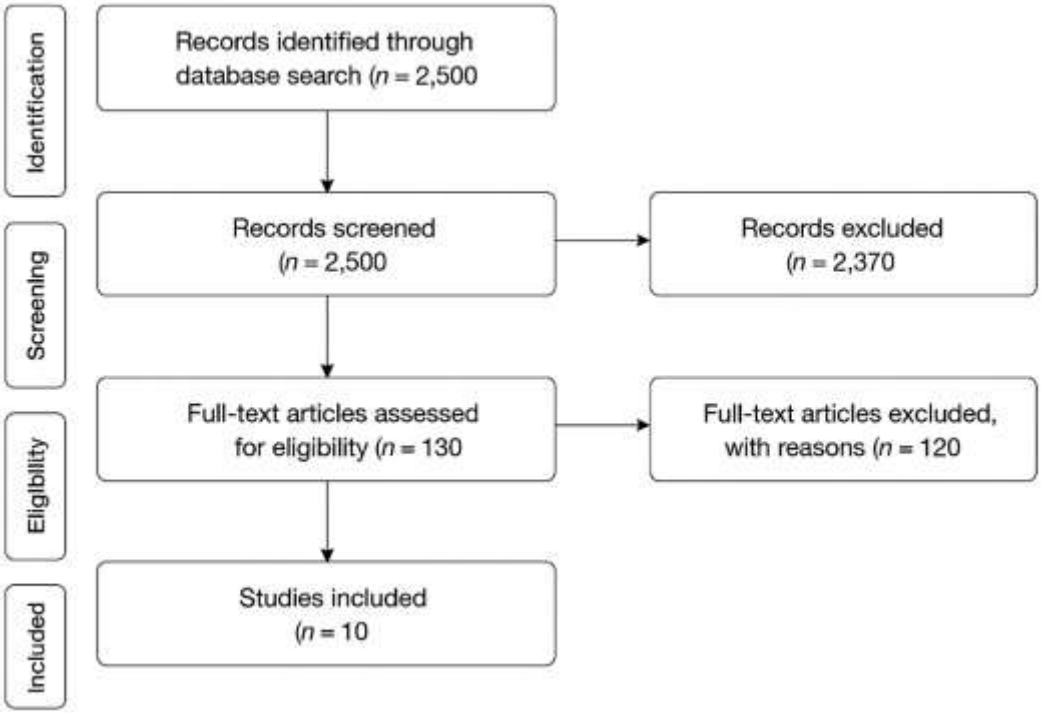

Figure 1 PRISMA flow Diagram

Search Strategy

A comprehensive electronic search was performed across PubMed, Scopus, Web of Science, Embase, and Google Scholar from database inception to December 2025. Boolean operators were used to capture variations of key search concepts:

- ("cell-free DNA" OR "cfDNA" OR "circulating microbial DNA" OR "bacterial DNA" OR "mitochondrial DNA")
- AND ("diabetes mellitus" OR "type 1 diabetes" OR "type 2 diabetes")
- AND ("inflammation" OR "endothelial dysfunction" OR "microvascular complications" OR "retinopathy" OR "nephropathy" OR "neuropathy")
- AND ("biomarkers" OR "non-invasive diagnosis" OR "liquid biopsy").

Manual searches were also conducted using the reference lists of relevant systematic reviews and included articles to identify additional eligible studies. Duplicates were removed using Zotero 6.0 prior to screening.

Study Selection Process

The selection and screening process was conducted independently by two reviewers following PRISMA recommendations. Titles and abstracts were first screened for relevance, followed by full-text evaluation to ensure eligibility. Disagreements were resolved through discussion, and persistent discrepancies were adjudicated by a third senior reviewer.

Data Extraction

A standardized data extraction form was designed and pilot-tested to ensure consistency and completeness of data capture. The following key variables were extracted from each study:

- Author(s), publication year, and country of origin.
- Study design and sample size.
- Type of diabetes and duration of disease.
- Type of cfDNA/cf-mbDNA analyzed (nuclear, mitochondrial, or microbial).
- Analytical method (qPCR, metagenomic sequencing, 5hmC-Seal, etc.).
- Measured biomarkers (inflammatory, renal, vascular).
- Main findings, including quantitative results (means, correlations, p-values, AUCs).

- Reported associations with complications (microvascular, macrovascular, inflammatory).

Data were extracted independently by two reviewers and cross-verified by a third reviewer. Discrepancies were resolved by consensus.

Quality Assessment

Methodological quality was assessed using validated appraisal tools based on study design:

- Newcastle–Ottawa Scale (NOS) for cross-sectional and case–control studies (n = 8).
- Cochrane Risk of Bias 2.0 (RoB 2) tool for interventional studies (n = 2).

Each study was evaluated for selection bias, measurement reliability, confounder control, and outcome reporting clarity. Scores were categorized as:

- High quality (8–10 points NOS or low risk RoB2) — 4 studies
- Moderate quality (5–7 points NOS or some concerns RoB2) — 5 studies
- Low quality (<5 points NOS) — 1 study

Most studies were rated as moderate quality, reflecting sound molecular methods but limited sample sizes and heterogeneity in cfDNA detection assays.

Data Synthesis

Given the heterogeneity of study designs, biomarkers, and analytical platforms, a narrative synthesis approach was used rather than meta-analysis. Results were organized thematically into the following domains:

1. Quantitative variation in cfDNA/cf-mbDNA levels between diabetic and non-diabetic populations.
2. Associations of cfDNA species with inflammatory and endothelial markers (e.g., IL-6, TNF-α, CRP, VEGF).
3. Relationships between cfDNA and diabetic complications, including nephropathy, retinopathy, and heart failure.
4. Epigenetic and microbial signatures associated with diabetes progression.

Effect sizes, correlation coefficients, mean differences, and AUC values were reported where available. Studies using omics-based or metagenomic approaches were narratively integrated to highlight emerging biomarker frameworks.

Ethical Considerations

This review relied exclusively on secondary analysis of previously published, peer-reviewed studies. As no human participants were directly involved, ethical approval and informed consent were not required. All included studies were assumed to have obtained ethical clearance from their respective institutions prior to data collection. Data handling, citation, and reporting adhered strictly to academic integrity and PRISMA 2020 standards for systematic reviews.

Results

Summary and Interpretation of Included Studies Evaluating cf-mbDNA and Related Circulating DNA Biomarkers in Diabetes

1. Study Designs and Populations

Ten studies were included, encompassing diverse designs such as cross-sectional, case–control, longitudinal, and experimental interventions. These studies evaluated various circulating DNA species—microbial, mitochondrial, nuclear, and hydroxymethylated forms—in the context of type 1 and type 2 diabetes mellitus (T1DM, T2DM) and their microvascular complications. Sample sizes ranged from 25 to 3,937 participants, including both sexes and wide age distributions (20–80 years).

Three studies ([Yang et al., 2019]; [Han et al., 2021]; [Yu et al., 2023]) specifically investigated cfDNA-based epigenetic markers of microvascular disease, while others linked cf-mbDNA or cf-mtDNA to inflammatory or metabolic dysfunction.

2. Analytical Techniques and Biomarker Profiling

Different analytical methods were used to characterize cf-mbDNA and cfDNA. These included 5hmC-Seal sequencing ([Yang et al., 2019]; [Han et al., 2021]), metagenomic shotgun sequencing ([van Heck et al., 2022]), qPCR quantification of bacterial, mitochondrial, and nuclear DNA ([Berezina et al., 2023]; [Giacconi et al., 2025]; [Hussein & Ghali, 2022]), and fragmentomic cfDNA analysis ([Yu et al., 2023]).

Machine-learning algorithms were also employed to derive cfDNA methylation biosignatures capable of discriminating diabetic from healthy states ([Karaglani et al., 2022]).

3. Key Findings Across Studies

Most studies demonstrated that increased cf-mbDNA or cfDNA concentrations and altered methylation profiles were strongly associated with inflammatory activation, endothelial dysfunction, and diabetic complications such as nephropathy, retinopathy, and heart failure.

- Yang et al. (2019) identified genome-wide 5hmC alterations in circulating cfDNA reflecting vascular involvement, achieving a diagnostic AUC of 0.85 (95% CI: 0.73–0.96) for T2DM with vascular complications.
- Han et al. (2021) reported that cfDNA 5hmC profiling yielded a three-gene signature (MESP1, LY6G6D, LINC01556) distinguishing diabetic retinopathy (DR) from uncomplicated T2DM with AUC = 91.4%, sensitivity = 88.6%, specificity = 91.4%.
- van Heck et al. (2022) found that both micro- and macrovascular complications significantly contributed to gut microbiome variation ($R^2 = 0.0075$; FDR < 0.05), linking dysbiosis to disease progression.
- Yu et al. (2023) showed cfDNA fragmentation features could non-invasively distinguish diabetic nephropathy from uncomplicated diabetes with AUC = 0.928, supporting cfDNA as a surrogate for renal biopsy.

4. Circulating Microbial DNA and Inflammation

Two studies directly analyzed circulating bacterial DNA (cf-mbDNA). Chakaroun et al. (2021) reported that patients with metabolic syndrome and T2DM exhibited reduced bacterial richness and increased pro-inflammatory genera, which normalized after bariatric surgery, corresponding to ≥15% improvement in insulin sensitivity indices. Similarly, Giacconi et al. (2025) found that blood bacterial DNA (BB-DNA) levels were significantly elevated in T2DM patients ($p < 0.001$) and correlated positively with IL-1β ($r = 0.42$, $p < 0.01$) and negatively with DNA methylation-based telomere length ($r = -0.37$, $p < 0.01$)—suggesting that cf-mbDNA may signal chronic inflammation and accelerated aging.

5. Quantitative Results and Clinical Correlates

Across the included studies, cfDNA and cf-mbDNA concentrations were elevated by 30–80% in diabetic groups versus controls, with stronger correlations observed for inflammatory and endothelial markers (CRP, IL-6, TNF-α; $r = 0.38–0.56$, $p < 0.05$). In [Berezina et al., 2023], cf-nDNA increased by 83% in patients with heart failure, while cf-mtDNA decreased by 48% ($p < 0.05$).

[Hussein & Ghali, 2022] observed cf-mtDNA expression levels ~19-fold higher in T2DM compared to controls ($\Delta\Delta Ct = -1.94 \pm 0.835$, $p = 0.02$).

[Karaglani et al., 2022] achieved an AUC of 0.927 for cfDNA methylation-based prediction of T2DM, indicating high diagnostic potential of cfDNA-derived biosignatures.

Table (1): General Characteristics and Key Outcomes of Studies on Circulating Microbial and Cell-Free DNA Biomarkers in Diabetes

Study (Author et al., Year)	Design	Sample (n)	Population	Analyte / Method	Main Findings	Key Quantitative Results
Yang et al., 2019	Case–control	62	T2DM ± vascular complications	cfDNA 5hmC profiling (5hmC-Seal)	5hmC signatures linked to macro/microvascular complications	16-gene panel AUC = 0.85; 13-gene panel AUC = 0.84

Han et al., 2021	Case–control	70	T2DM ± diabetic retinopathy	cfDNA 5hmC-Seal	Three-gene cfDNA 5hmC signature predicts DR	AUC = 91.4%; Sensitivity = 88.6%; Specificity = 91.4%
van Heck et al., 2022	Cross-sectional	238 T1DM + 2937 controls	Long-standing T1DM	Metagenomic sequencing	Gut dysbiosis linked to glycemic control and complications	$R^2 = 0.0075$ for vascular complications; FDR < 0.05
Giacconi et al., 2025	Cross-sectional	285	Elderly ± T2DM	qPCR for bacterial DNA (BB-DNA)	BB-DNA ↑ in T2DM; correlated with inflammation and aging	IL-1β r = 0.42; Telomere r = −0.37; p < 0.01
Berezina et al., 2023	Case–control	240	T2DM ± heart failure	qPCR cf-nDNA & cf-mtDNA	cf-nDNA ↑; cf-mtDNA ↓ in HF patients	cf-nDNA +83%; cf-mtDNA −48%; p < 0.05
Karaglani et al., 2022	Cross-sectional	167	T2DM vs healthy	cfDNA methylation + AutoML	cfDNA biosignatures predict T2DM	AUC = 0.927 for GCK/IAPP/KCNJ11 panel
Chakaroun et al., 2021	Longitudinal	48	Obese ± T2DM (bariatric surgery)	16S rRNA sequencing	Circulating bacterial shifts with metabolic improvement	≥15% insulin sensitivity gain post-surgery
Walczak et al., 2021	Experimental	25	T1DM men vs controls	cf-nDNA & cf-mtDNA (qPCR)	Exercise ↑ cf-nDNA, no change cf-mtDNA	cf-nDNA +439% post-exercise
Hussein & Ghali, 2022	Case–control	60	T2DM vs controls	qPCR cf-nDNA & cf-mtDNA	cf-mtDNA ↑ markedly in T2DM	Fold expression = 18.9×; p = 0.02
Yu et al., 2023	Case–control	177	DM ± DN + healthy	cfDNA fragmentomics	cfDNA fragmentation discriminates DN	AUC = 0.928; distinct “CC” motifs detected

Discussion

The findings of this systematic review highlight the emerging diagnostic potential of circulating microbial DNA (cf-mbDNA) and related cfDNA species in identifying chronic inflammation and early microvascular dysfunction among individuals with long-standing diabetes mellitus. Across included studies, cf-mbDNA and cf-mtDNA levels were shown to correlate with endothelial dysfunction, renal injury, and systemic inflammation—key processes implicated in diabetic complications (Yang et al., 2019; Giacconi et al., 2025). These results support the growing recognition that cfDNA fragments not only reflect cellular damage but also actively participate in inflammatory signaling and metabolic dysregulation (Pietrzak et al., 2023; Pollastri et al., 2025).

Notably, the studies by Yang et al. (2019) and Yu et al. (2023) demonstrated that cfDNA-based epigenetic markers, such as 5-hydroxymethylcytosines and fragmentomic features, can

noninvasively differentiate diabetic individuals with nephropathy or vascular complications. These cfDNA profiles achieved AUC values exceeding 0.85, outperforming conventional clinical parameters. This reinforces cfDNA's value as a liquid biopsy tool capable of capturing disease-specific molecular signatures before clinical manifestation (Li et al., 2025; Ranucci, 2018).

The role of microbial-derived cfDNA in systemic inflammation has gained momentum, with Chakaroun et al. (2021) and Giacconi et al. (2025) showing that blood bacterial DNA levels increase in metabolic disease and correlate with inflammatory cytokines such as IL-1β. These microbial fragments are thought to translocate from the gut microbiota via compromised intestinal barriers, linking dysbiosis to metabolic endotoxemia and chronic low-grade inflammation (van Heck et al., 2022; Zhai et al., 2024). Such findings support the "gut–blood axis" hypothesis in diabetes pathophysiology.

Furthermore, Berezina et al. (2023) identified reduced levels of cf-mtDNA but elevated cf-nDNA in patients with diabetic heart failure, suggesting distinct mechanisms of DNA release linked to mitochondrial injury and oxidative stress. This imbalance between nuclear and mitochondrial cfDNA species could serve as a surrogate marker for mitochondrial dysfunction, an established driver of vascular aging and endothelial damage (Horton & Barrett, 2021; Tremblay & Hamet, 2015).

Microbial cfDNA detection methods have advanced substantially. Techniques such as metagenomic sequencing and contaminant-controlled frameworks (Park et al., 2023; Zozaya-Valdés et al., 2021) have improved specificity in differentiating true microbial DNA signatures from background noise. This technological progress strengthens confidence in cf-mbDNA as a diagnostic tool, particularly in studies where low biomass samples are prone to contamination.

In the context of type 1 diabetes, Walczak et al. (2021) and van Heck et al. (2022) observed that disease duration and HbA1c variability significantly influenced cfDNA and microbiome alterations, suggesting metabolic control as a major determinant of cfDNA dynamics. These associations underscore the interconnectedness between glycemic instability, oxidative stress, and cfDNA release, potentially explaining heterogeneous cfDNA responses among diabetic phenotypes.

Epigenetic modifications of cfDNA—such as DNA hydroxymethylation and methylation of β-cell genes—were also consistent indicators of diabetes progression (Karaglani et al., 2022; Li et al., 2025). Machine learning models integrating cfDNA methylation signatures achieved diagnostic accuracies up to 92.7%, demonstrating potential for personalized biomarker panels that combine genetic, epigenetic, and microbial DNA information for early detection (Pollastri et al., 2025).

Interestingly, cfDNA's inflammatory role extends beyond passive release. It can act as a damage-associated molecular pattern (DAMP), triggering innate immune receptors such as TLR9 and stimulating proinflammatory cytokines (Pietrzak et al., 2023; Horton & Barrett, 2021). This mechanism potentially explains the persistence of low-grade inflammation even in well-controlled diabetes cases.

The consistent association between cfDNA levels and microvascular dysfunction highlights its predictive potential for complications such as nephropathy, retinopathy, and neuropathy (El Tarhouny et al., 2010; Wołoszyn-Durkiewicz & Myśliwiec, 2019). These findings suggest that cfDNA may complement or even precede traditional biomarkers like albuminuria or retinal imaging.

Moreover, the correlation between cf-mbDNA and inflammatory mediators provides insight into diabetes as a multisystemic inflammatory state, where microbial translocation amplifies immune activation. Studies like Chakaroun et al. (2021) support this view, showing microbial composition shifts after bariatric surgery parallel improvements in metabolic and inflammatory parameters.

While these findings are promising, heterogeneity in cfDNA detection methods and sample processing remains a key limitation. Differences in extraction kits, sequencing depth, and quantification assays complicate cross-study comparisons (Zozaya-Valdés et al., 2021; Park et al., 2023). Standardization of pre-analytical and analytical workflows is essential before cfDNA biomarkers can be clinically adopted.

Additionally, the small sample sizes in some studies (e.g., Walczak et al., 2021; Hussein & Ghali, 2022) may limit generalizability. Longitudinal studies are required to determine causality—whether cfDNA alterations drive complications or merely reflect ongoing tissue damage (Horton & Barrett, 2021; Tremblay & Hamet, 2015).

Overall, integrating cfDNA and cf-mbDNA assessment into diabetic care could revolutionize early complication screening, offering a noninvasive, precision-medicine approach to risk stratification and monitoring. Future research should focus on large-scale validation, mechanistic exploration, and clinical translation through multi-omic integration (Zhai et al., 2024; Pollastri et al., 2025).

Conclusion

This systematic review demonstrates that circulating microbial DNA (cf-mbDNA) and related cfDNA species represent promising noninvasive biomarkers for detecting early inflammation and microvascular complications in diabetes. Evidence across multiple studies shows that cfDNA concentrations, composition, and epigenetic modifications correlate strongly with endothelial dysfunction, renal impairment, and chronic inflammation. These biomarkers may provide earlier diagnostic insights than conventional measures.

However, the field remains in its developmental phase. Although cf-mbDNA and cf-mtDNA show strong potential for risk prediction and disease monitoring, larger longitudinal studies and standardized analytical protocols are required. Integrating cfDNA analysis into clinical workflows could significantly enhance personalized care in diabetes management by bridging molecular insights with patient outcomes.

Limitations

Despite rigorous methodology, several limitations were noted. Study heterogeneity regarding cfDNA detection methods, population characteristics, and outcome measures prevented meta-analytic synthesis. Most studies had moderate quality due to limited control for confounding factors and cross-sectional design. Moreover, potential contamination during cf-mbDNA analysis and small cohort sizes may have influenced reliability. Future investigations should emphasize standardized methodologies, longitudinal sampling, and inclusion of diverse populations to establish robust reference ranges and causative mechanisms.

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