Review Article

Early Detection of Diabetic Nephropathy Based on Urinary and Serum Biomarkers: An Updated Systematic Review

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Abstract

Diabetic nephropathy (DN) is a leading cause of chronic kidney disease (CKD) and end-stage renal disease worldwide, particularly among individuals with type 2 diabetes mellitus (T2DM). Early detection and intervention are crucial in slowing the progression of DN and improving patient outcomes. Traditional diagnostic methods, such as the measurement of albuminuria and serum creatinine, often fail to detect early renal damage because structural kidney damage may occur before albumin excretion. This systematic review aims to evaluate the diagnostic value of various urinary and serum biomarkers in the early detection of DN in patients with T2DM. A comprehensive literature search was conducted using databases such as PubMed, Scopus, and Web of Science. We only considered studies involving human populations for inclusion in our analysis. Animal and *in vitro* studies were excluded from our review. Our analysis of 17 observational studies identified several key serum biomarkers, such as netrin-1, osteopontin, adiponectin, and specific cytokines (e.g., IL-6, IL-8), which show significant promise for early detection of DN. Urinary biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL), transferrin, N-acetyl-β-D-glucosaminidase (NAG), and various cytokines, have also proven to be reliable indicators. The combination of both serum and urinary biomarkers may enhance diagnostic accuracy and enable earlier intervention. Additionally, incorporating genetic and mRNA markers could provide a more comprehensive approach to early DN detection. Implementing these biomarkers in clinical practice could significantly improve outcomes for patients with DN by facilitating early diagnosis and timely management.

Keywords: Biomarkers, diabetes, diabetic nephropathy, review

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Introduction

Type 2 diabetes (T2D) or diabetes mellitus (DM) is a noncommunicable chronic disease with a high rate of all-cause morbidity and mortality and severe outcomes such as kidney failure, cardiovascular disease (CVD), and diabetic retinopathy.[1,2] Diabetic nephropathy (DN) is the leading cause of chronic kidney failure, starting with albuminuria and ultimately leading to end-stage renal disease (ESRD).[3]

Proteinuria has long been considered the gold standard for assessing and tracking renal function, alongside current GFR and past GFR trajectory. However, proteinuria alone is insufficient to monitor the progression of DN as renal function deteriorates in approximately one-third of patients before the onset of albuminuria.[4-6] On the other hand, factors such as exercise, infection, severe comorbidities, and cardiac failure can confound albuminuria measurements. Furthermore, albuminuria can also be present in individuals without diabetes, highlighting its lack of specificity for accurately predicting DN.[7,8] In addition, some individuals without microalbuminuria exhibit advanced renal pathological changes.[5,9]

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Currently, no biomarkers are available that can predict whether individuals with T2D will develop more severe kidney disease.[10] Consequently, additional laboratory biomarkers are needed for the early diagnosis of DN, even before the onset of microalbuminuria, to enable earlier medical interventions and delay kidney failure. Recent research has identified several novel and significant urinary biomarkers that can predict DN earlier and with greater specificity.[6,11,12] These biomarkers reflect kidney injury as a result of oxidative stress, glomerular and tubular damage, renal inflammation, urinary exosomes, and microRNAs (miRNAs).[6,13-19]

While various literature reviews have been conducted to introduce new diagnostic serum or urine biomarkers that can be used to screen for DN,[6,20-22] conducting a systematic review on this topic is essential for consolidating existing knowledge, addressing gaps, and providing practical clinical guidance. This comprehensive approach can significantly enhance the early diagnosis and management of DN, ultimately improving patient outcomes.

Materials and Methods

The study selection process adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[23] All the steps including searching, selection of final included papers, and quality assessment of articles were performed by authors independently, and any discrepancies were resolved through discussion or consultation with a third reviewer.

Search strategy

We conducted a literature review using PubMed, Google Scholar, and WOS to see if urinary biomarkers or serum biomarkers could detect early DN more effectively. The following keywords were searched individually or in conjunction with others: "diabetic nephropathy", "biomarkers", "urinary", "serum", "plasma", and "diagnostic" in the title, keywords, and abstract. The search terms were connected by 'AND' and 'OR' Boolean operators.

Inclusion and exclusion criteria

We included cross-sectional, cohort, and case-control studies published in English from January 2000 to September 2022. These studies involved adult participants (aged 18 years and older) diagnosed with T2DM. Additionally, we ensured that the studies provided sufficient data on diagnostic performance and met quality appraisal standards. Studies with nonrelated topics or nonsufficient reported data, reviews, abstracts, and articles that were not in English were excluded. We only considered studies involving human populations for inclusion in our analysis. Animal and *in vitro* studies were excluded from our review.

Study selection

The articles that were found were exported to EndNote Reference Manager, and subsequently, duplicates were removed. Based on the eligibility criteria, two independent reviewers screened the articles by title and abstract, followed by full-text screening. During the study selection process, disagreements were resolved through consensus from the third reviewer.

Data extraction

The data from the selected studies were extracted based on a prespecified data extraction form, which included the following data variables: author, year, country of study, study design, study population, details of the type of biomarkers (urinary or serum), findings, and comparison of diagnostic value of serum biomarkers versus urinary biomarkers.

Results

The initial database search yielded a total of 1226 articles. Figure 1 illustrates the comprehensive study selection process. After removing duplicates and screening titles and abstracts, 17 studies were ultimately included based on our inclusion criteria. Since all the included studies were observational, their methodological quality was assessed by the nine-star Newcastle Ottawa Scale (NOS),[24] which consists of three major aspects: selection, comparability, and exposure or outcome. All studies were scored with 7 or more stars and considered as high quality. Table 1 includes characteristics and outcomes extracted from included studies.

Discussion

DN remains a significant complication of diabetes mellitus, and early detection is crucial for preventing progression to end-stage renal disease. Various studies have investigated serum and urinary biomarkers to identify early indicators of DN across different populations and geographical regions. This systematic review explored a comparison between urinary biomarkers and serum biomarkers in the early detection of renal damage, associated with DN in T2D. We gathered 17 articles that looked at the efficacy of serum and urine biomarkers in the human population [Table 1].

The collected data from these 17 studies illustrate the potential of various biomarkers for diagnosing DN at different stages of its progression. Early markers such as netrin-1, osteopontin, and FABP4 are crucial for early intervention, while others like advanced glycation end products, superoxide dismutase, and β2-microglobulin are more relevant for later stages. The integration of these biomarkers into clinical practice could greatly enhance the ability to detect DN early, allowing for timely and targeted management strategies.

Serum biomarkers

Several studies have highlighted the efficacy of serum biomarkers in detecting early DN. For instance, Al-Rubeaan *et al*. (2017)[25] found that serum osteopontin showed promise as an early DN biomarker in a Saudi Arabian cohort. Similarly, Elkholy *et al*. (2021)[26] demonstrated that serum netrin-1 was effective in an Egyptian population. Harkin *et al*. (2022)[10] identified multiple serum markers, including adiponectin and

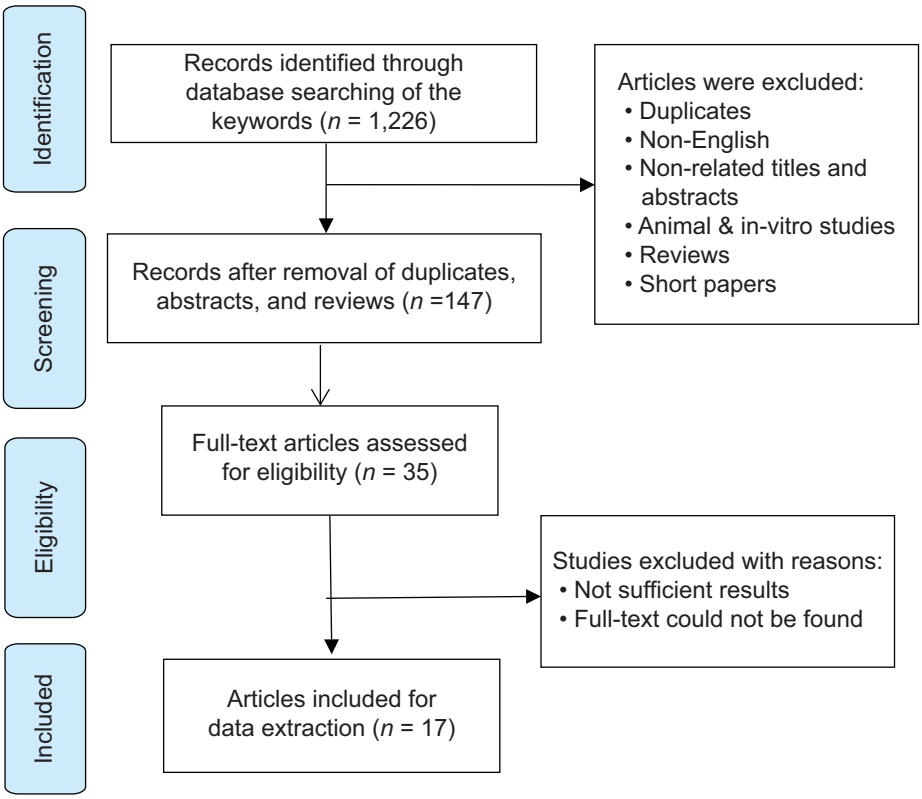

Figure 1: Flowchart of study selection

CRP, as significant in a UK cohort. Additionally, studies from China by Zhou *et al*. (2018)[30] and Wang *et al*. (2015)[35] found that lower DHA and higher β2-microglobulin and increased osteoinductive factor (OIF), respectively, correlated with DN progression.

Urinary biomarkers

Urinary biomarkers also proved useful across various studies. Al- Rubeaan *et al*. (2017)[25] reported that urinary transferrin, NGAL, and RBP were effective in identifying early DN. Zhang *et al*. (2019)[29] supported these findings, particularly highlighting urinary transferrin and NGAL. Uslu *et al*. (2005)[28] from Turkey found that urinary NAG, ALP, and LDH were significant markers. Moreover, Ni *et al*. (2018)[36] demonstrated that urinary albumin-to-creatinine ratio (UACR) and cystatin C-to-creatinine ratio (CysC/Cr) were effective indicators of DN in a Chinese population.

Combined biomarkers

Combining serum and urinary biomarkers can enhance the predictive power for early DN detection. Kamel *et al*. (2022)[27] noted the potential of combining serum zinc alpha 2 glycoprotein with urinary adiponectin, transferrin, and RBP. Lin *et al*. (2023)[37] further demonstrated that a combination of urinary NAG and serum RBP provided strong predictive value for DN.

Genetic and mRNA markers

Explorations into genetic and mRNA markers also revealed potential for early DN detection. Hashemi *et al*. (2021)[31] highlighted WT1 and ACE mRNAs as effective early markers. Veiga *et al*. (2020)[39] and Al-Hazmi *et al*. (2020)[33] emphasized the significance of NGAL gene expression in both serum and urine.

Advantages of using the diagnostic biomarkers compared to proteinuria

The biomarkers introduced in this study offer several advantages over traditional proteinuria measurement for the early detection and management of DN:

Early Detection: Netrin-1, Osteopontin, FABP4, and Other Cytokines (IL-6, IL-8): These biomarkers can detect DN earlier than proteinuria, often before any significant albumin is present in the urine. This early detection allows for timely intervention to prevent or slow disease progression.

Sensitivity and Specificity: NGAL, Cystatin C, and adiponectin: These biomarkers provide higher sensitivity and specificity compared to proteinuria. They can detect subtle changes in renal function and inflammation, offering more precise diagnostic information.

Reflect Different Pathophysiological Processes: Cytokines (IL-6, IL-1a, IL-18, TNF-α), Advanced glycation end products (AGEs), and superoxide dismutase: These markers provide insights into different aspects of DN pathophysiology, including inflammation, oxidative stress, and cellular injury, whereas proteinuria primarily reflects glomerular damage.

Table 1: Extracted data and summary of the results

Study/year/country	Type of Study	population	Serum/plasma biomarkers	Urinary biomarkers	Results/indication of the presence of DN
Al-Rubeaan *et al*., 2017/Saudi Arabia[25]	cross-sectional	467 DN patients	IL-6, IL-1a, IL-18, MCP-1, TNF-α, L-selectin, E-selectin, P-selectin, Fetuin A, Osteopontin, Cys C	Transferrin, NGAL, RBP	Urinary transferrin, NGAL, RBP, and serum osteopenia showed good results as early DN biomarkers
Elkholy *et al*. 2021/ Egypt[26]	Case-Control	135 patients with type 2 DM 45 healthy subjects as control	serum netrin-1	urinary netrin-1	Serum netrin-1 showed good results as early DN biomarkers
Harkin *et al*. 2022/ UK[10]	Case-Control	62 patients with type 2 DM 26 healthy subjects as control	Adiponectin, CRP, Cystatin C, IFN-ɣ, NGAL, IL-2, IL-4, IL-8, IL-10	IL-4, IL6, IL-8, KIM-1, MCP1, Microalbumin, Midkine, MIP1	Serum biomarkers showed good results as early DN biomarkers
Kamel *et al*. 2022/ Egypt[27]	Case-Control	60 patients with type 2 DM 20 healthy subjects as control	Zinc Alpha 2 Glycoprotein	adiponectin, transferrin, RBP	Serum and urine biomarkers showed good results as early DN biomarkers
Uslu *et al*. 2005/ Turkey[28]	cross-sectional	56 patients with type 2 DM 20 healthy subjects as control	Cys C, HbA1C	NAG, ALP, LDH	Serum Cys C levels and urinary NAG, ALP, and LDH activities showed good results as early DN biomarkers
Zhang *et al*. 2019/ China[29]	Case-Control	287 patients with T2DM & DN, normoalbuminuria (*n*=144), microalbuminuria (*n*=94), macroalbuminuria (*n*=49) healthy controls (*n*=42)	Cys C, total bilirubin, uric acid	Transferrin, IgG, NGAL, NAG, TNFα, IL-18	Urinary transferrin, TNFα, IgG, NGAL, and the combination of all four biomarkers showed good results as early DN biomarkers
Zhou *et al*. 2018/ China[30]	Case-Control	100 T2DM patients 3 groups of non-DN, early DN, and clinical DN	DHA, advanced glycation end products, fractalkine, superoxide dismutase, TNFα	HbA1C, β2-microglobulin	Lower serum DHA and superoxide dismutase and higher serum β2-microglobulin showed good results as early DN biomarkers
Hashemi *et al*. 2021/ Iran[31]	cross-sectional	103 patients with DN 100 patients with type 2 DM 53 healthy subjects as control		WT1 mRNA, ACE mRNA	WT1 and ACE mRNAs level showed good results as early DN biomarkers
Siddiqi *et al*. 2017/ India[32]	cross-sectional	90 patients with type 2 DM 90 healthy subjects as control	NGAL, Cys C		NGAL, Cys C showed good results as early DN biomarkers
Al-Hazmi *et al*. 2020/ Saudi Arabia[33]	cross-sectional	86 patients with T2DM	NGAL, NAG, cystatin C		NGAL showed good results as early DN biomarkers
Assal *et al*. 2013, Egypt[34]	Cohort	70 patients with T2DM	NGAL, NAG, cystatin C		NGAL showed good results as early DN biomarkers
Wang *et al*. 2015, China[35]	cross-sectional	90 patients with type 2 DM 30 healthy subjects as control	Osteoinductive factor (OIF)		OIF is a significant marker for early detection of DN. OIF levels increase with progression of DN.
Ni *et al*. 2018, China[36]	cross-sectional	172 patients with type 2 DM 20 healthy subjects as control	Serum fatty acid-binding protein 4 (FABP4)	albumin-to-creatinine ratio (UACR) cystatin C-to-creatinine ratio (CysC/Cr)	Serum FABP4 is a biomarker for early DN detection. FABP4 levels correlate with albuminuria and GFR in diabetic patients.

Contd...

Table 1: Contd...

Study/year/country	Type of Study	population	Serum/plasma biomarkers	Urinary biomarkers	Results/indication of the presence of DN
Lin *et al*. 2023, China[37]	retrospective	50 patients with type 2 DM	serum retinol-binding protein (RBP)	Urinary NAG	Urinary NAG and serum RBP are potential biomarkers for DN. Combination of urinary NAG and serum RBP predicts DN early.
Omozee *et al*. 2019, Nigeria[38]	cross-sectional	56 patients with type 2 DM 30 healthy subjects as control		Urinary NAG	Urinary NAG is an early biomarker for DN detection. Urinary NAG levels correlate with DN progression
Veiga *et al*. 2020, Brazil[39]	cross-sectional	51 patients with type 2 DM 39 healthy subjects as control	Gene expression of NGAL, type IV collagen (COLIV1A), and SMAD1	Gene expression of NGAL, type IV collagen (COLIV1A), and SMAD1	NGAL and SMAD1 gene expression in blood and urine samples are potential early biomarkers for DN.
Liao *et al*. 2018, Taiwan[40]	cross-sectional	40 patients with type 2 DM 35 healthy subjects as control		Urinary HPT and AMBP	haptoglobin (HPT) and α-1-microglobulin/bikunin precursor (AMBP) showed good results as early DN biomarkers

T2DM: Type 2 diabetes mellitus; DN: diabetic nephropathy; WT1 gene: tumor suppressor gene wt1 (Wilms' tumor gene); ACE mRNA: angiotensin-converting enzyme (ACE) mRNA; CRP: C-reactive protein; Cystatin C: Serum cystatin C; IFN-γ: IFN-gamma; IL: Interleukin; RBP: Retinol Binding Protein, HbA1C : Glycated hemoglobin; NGAL: neutrophil gelatinase-associated lipocalin; IgG: immunoglobulin G; TNFα: tumor necrosis factor-α; NAG: N-acetyl-b-D-glucosaminidase; ALP: alkaline phosphatase; LDH: Lactate dehydrogenase; KIM1: Kidney Injury Molecule-1; MCP1: monocyte chemoattractant protein-1; MIP1: Macrophage inflammatory protein-1 alpha; OIF: Osteoinductive factor; FABP4: Serum fatty acid-binding protein 4; UACR: albumin-to-creatinine ratio; CysC/Cr: cystatin C-to-creatinine ratio; RBP: serum retinol-binding protein; SMAD1: suppressor of mothers against decapentaplegic type 1; HPT: haptoglobin; AMBP: α-1-microglobulin/bikunin precursor

Non-Invasive and Convenient: urinary Biomarkers (NGAL, transferrin, NAG): Collecting urine samples for these biomarkers is noninvasive and can be more convenient for patients, potentially leading to better compliance in regular monitoring compared to blood tests.

Combination of Biomarkers: serum and urinary NAG, RBP, and gene expression markers (NGAL, SMAD1): using a combination of serum and urinary biomarkers can enhance diagnostic accuracy, providing a more comprehensive overview of renal health and allowing for better risk stratification and personalized treatment plans.

Correlation with Disease Progression: serum and urinary NAG, alkaline phosphatase (ALP), lactate dehydrogenase (LDH): these biomarkers not only aid in early detection but also correlate well with disease progression, enabling ongoing monitoring of disease status and treatment efficacy.

Improved clinical outcomes: Osteoinductive Factor (OIF), Haptoglobin (HPT), and α-1-microglobulin/bikunin precursor (AMBP): these biomarkers can guide early therapeutic interventions, which may improve long-term renal outcomes and reduce the incidence of ESRD.

Conclusion

The studies reviewed indicate robust potential for various serum and urinary biomarkers in the early detection of DN. Key serum biomarkers such as netrin-1, osteopontin, adiponectin, and specific cytokines (e.g., IL-6, IL-8) have shown significant promise. Urinary biomarkers, including NGAL, transferrin, NAG, and various cytokines, also provide reliable early detection methods. Combining both serum and urinary biomarkers may enhance diagnostic accuracy and allow for early intervention strategies.

Limitations

Given the diversity in populations and methodologies across the studies, further research should focus on standardizing biomarker assessment protocols and validating these markers in larger, multicenter cohorts.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Availability of data and materials

All data generated during this study are included in this published article.

Author contributions

Farzaneh Karimi contributed to the study conception and design. The first draft of the manuscript was written by Farzaneh Karimi and Negar Dinarvand, all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest

There are no conflicts of interest.

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