



## Proteomic Biomarkers as Early Detection of Diabetic Nephropathy

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### ABSTRACT

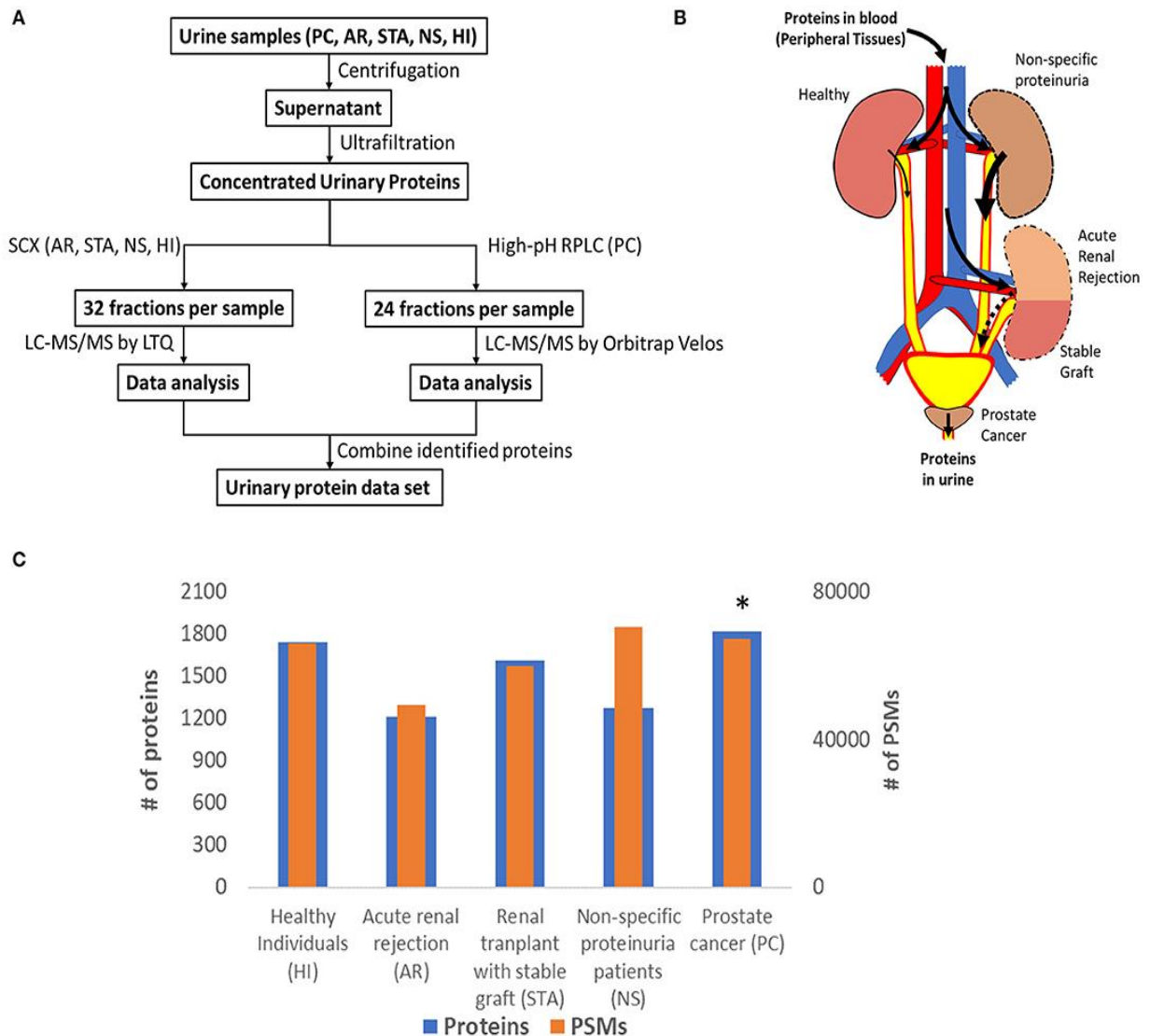
Diabetic nephropathy (DN), which results in chronic kidney disease (CKD) and necessitates dialysis and renal transplantation, is a typical consequence of long-term diabetes. The best DN predictor now available in the clinic is microalbuminuria, which is regarded as a risk marker suggesting the potential start of DN, making it a marker rather than a predictor. New indicators are therefore necessary for early diagnosis and DN therapy. Several Capillary electrophoresis coupled to mass spectrometry (CE-MS) was used to investigate urine proteins and has various benefits, a main advantage is reproducibility. In recent years, numerous urine proteins have been examined to learn further about their potential as DN indicators. Although their nature and regulation differed across investigations, collagen fragments were discovered in various proteome analyses. Diabetic patient urine also comprised more serum albumin fragments, a transthyretin fragment, and peptides with an additional alteration (oxidation). The present review's aim is to screen for analysis of proteomics for their identity and quantity, which enable the identification of new biomarkers and early detection of DN with promising clinical value.

**Keywords:** *Diabetic Nephropathy- Chronic Kidney-diagnostic proteomics-Mass spectrometry*

### 1. Introduction

Diabetic nephropathy (DN), a common consequence of long-term diabetes, causes chronic kidney disease (CKD), which may require dialysis and subsequently a kidney transplant (Drugge H, 2013). Urinary proteins may come from distant organs and tissues as well as the kidney, bladder, prostate gland, ureter, and urethra (Ploussard G, 2009). Usually, it begins slowly and asymptomatic but grows, leading to renal function deterioration. It is a significant public health issue that may be addressed early on with more precise and non-invasive techniques (Levey A, 2007). The prevention of end-stage renal disease (ESRD) in diabetic patients by delaying and, if possible, interrupting the progression of renal damage is one of the key objectives of early detection of DN. The avoidance of end-stage renal disease (ESRD) in diabetic patients by delaying and, if possible, interrupting the progression of renal damage is one of the key objectives of early detection of DN. Even though they're not a prediction, routine diagnostic tests are valuable for determining the onset, continuation, and responsiveness to therapeutic interventions (Suarez M, 2013). High inter-individual variability and, as a result, poor specificity and sensitivity at the early stages of the disease are common obstacles to early diagnosis with these standardized tests. Even though these tests are beneficial to patients with advanced DN (Miller W, 2009). In general, the evolution of DN is characterized by accelerating increase of urinary albumin excretion rate (UAER), which is defined as more than 30 mg/gm creatinine in spot urine

test or 24-hour urine (American Diabetes Association,2013).



**Figure 1.** The relative number of proteins and PSMs across situations; (A) a description of the procedure for urine proteome analysis; (B) an illustration of probable sources of protein biomarkers from various organs in the urine; and (C) an overview of the workflow for urine proteome analysis. Note that to account for the variances in MS platforms, the PSM numbers for PC were normalized against those for HI (Swensen AC,2021).

## 2. Diagnosis of DN:

The best DN predictor now available in the clinic is microalbuminuria, which is regarded as a risk marker for the beginning of DN (Rossing P,2006). Microalbuminuria may also be a sign rather than a predictor of DN due to the fact that it is neither specific for DN nor highly variable within an individual (Macisaac R,2011). It was initially thought, that DN would emerge in 80% of persons with type 1 diabetes and microalbuminuria, however, prior research has shown that only 34% of these patients proceed to macro albuminuria less than 20%

even revert to normoalbuminuria (Hovind P,2004). Additionally, glomerular hyperfiltration has no impact on the emergence of microalbuminuria in Diabetes type1 after 15 years from diagnosis (Ficociello L,2009). Furthermore, in diabetic patients with newly developed microalbuminuria, the progression to proteinuria may not be necessary for the development of progressive chronic kidney disease (Perkins B,2010).

At epidemiologic scales, microalbuminuria increases the proportional risk of developing diabetic and non-diabetic nephropathy. Additionally, even within the "normal" range, urine albumin excretion levels are linked to an elevated risk of cardiovascular endpoints in those without diabetes. The ACR (albumin to creatinine ratio) of 30 mg/g, which is used to define "normoalbuminuria," is actually fairly arbitrary and does not always correspond to a typical value as defined by statistics." Thus, the level of risk for ACR varied based on whether cardiovascular risk or risk for renal illness is taken into account. Therefore, enhanced diagnostic methods, screening tools, and indicators are required for diabetic nephropathy. The normal ACR is probably going to be classified as an abnormal test with a cutoff of 10 mg/g and utilized primarily as a marker for endothelial dysfunction (Roshan B,2013).

### 3. Early monitoring of peptides

Urinary protein and peptide profiling methods based on mass spectrometry can reveal changes in the excretion rates of particular proteins and peptides that have predictive value in the clinical era, such as early disease diagnosis, disease classification based on likely therapeutic responses, prognosis assessment, and monitoring response to therapy. These methods may be useful for treating systemic disorders linked to circulating peptide and small protein indicators that can pass the glomerular filter (Pisitkun T,2006).

Proteomics using mass spectrometry (MS) offers an effective analytical method for the widespread identification of proteins in urine. This is required to make it easier to find new urine protein biomarkers. The creation of a comprehensive urine protein database should make use of samples from both sick and healthy people (Swensen AC,2021). While clinical laboratories will benefit from using mass spectrometers to assess biomarkers, there is a major risk that doing so will result in higher expenses and the need to purchase additional equipment. However, the creation of trustworthy assays for illness biomarkers in urine has the potential to result in a net drop in overall expenditures. An early diagnosis of glomerular disease, for instance, can enable therapeutic interventions that significantly postpone or prevent the onset of end-stage renal disease, hence lowering the need for dialysis and transplantation. (Perkins B,2010).

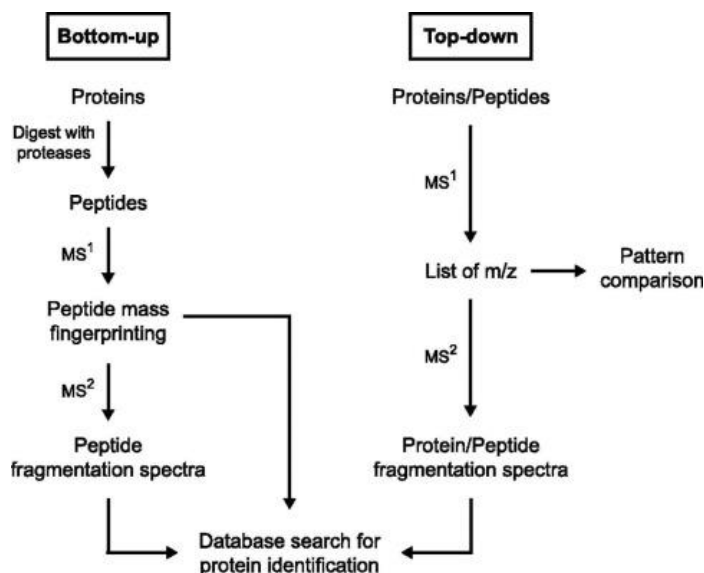


Fig. 3. **Schematic of top-down versus bottom-up approaches.** Small proteins and/or peptides are typically treated using the top-down method without first undergoing digestion. In this approach, the list of m/z that may be utilised for pattern comparison between control and illness states is produced by the first stage mass

analysis of precursor ions (MS1). Contrarily, the bottom-up method necessitates a preliminary step of protease digestion to fragment proteins into peptides, enabling protein identification based on peptide mass fingerprinting from MS1 or peptide fragmentation spectra from MS. (Pang J.X, 2002).

New diagnostic tools are thus required for early DN identification and treatment. In years, a series of urine proteins have been examined to determine whether they can serve as DN predictors (Matheson A, 2010). A major benefit of capillary electrophoresis linked to mass spectrometry (CE-MS) over other proteomic techniques is its high repeatability (Mischack H, 2009). Recent investigations at various institutions have employed CE-MS to examine urine samples from healthy volunteers and patients with a range of renal disorders (Decramer S, 2006).

A complex mixture of whole proteins or particular protein fragments resulting from urine resident serum proteins or renal parenchymal proteins are likely to make up the biomarkers of renal disorders characterized by proteinuria (Merchant M, 2010).<sup>1</sup> Soluble proteins and protein parts of urine's solid phase components are both examples of urinary proteins. Exosomes, which are very low density (80-nm) vesicles that exist as internal vesicles in multivesicular bodies and sediment develop only with ultracentrifugation, are made up of "sediments" that can be precipitated at low centrifugation speeds. According to research on urine samples taken from healthy adult human volunteers, of the total urinary protein expelled, 48% was found in sediments, 49% was soluble, and the final 3% was found in exosomes (Zhou H, 2006).

The majority of the soluble proteins in urine come from glomerular filtration. Small proteins (10 kDa) which the glomerulus freely filtered, whereas high molecular weight proteins (eptides) are efficiently blocked from passing through the glomerular filter. In the proximal tubule, the majority of the proteins and peptides that pass the glomerular filter are scavenged and proteolyzed by highly specialised apical absorption processes that entail receptor-like identification of the polypeptide molecules. Therefore, a change in a certain soluble protein's concentration in blood plasma, a change in the glomerular filter's operation, or a change in the proximal tubule scavenging system can all affect how much of that protein ends up in the final urine. Using these techniques, it is possible to determine if variations in the excretion rate of particular urine proteins are a sign of systemic diseases, glomerular disease, or pathologies of the proximal tubule, respectively (Christensen E, 2002).

Solid phase components—Urine frequently contains sediments of a very high density that are primarily made up of casts and dead epithelial cells, both of which can be separated using a centrifuge at a low speed. Renal illness may be indicated by an increase in whole cells or casts or the classification of cells and casts based on microscopy crucial diagnostic information. A source of urinary proteins and a possible source of biomarkers which can be found in solid-phase components of urine. Some of these parts are tiny membrane fragments that are transported to the urinary area by apoptosis or microvilli shedding (Pisitkun T, 2004).

#### 4.Role of Proteomics:

Several proteomic investigations revealed fragments of collagen, albeit the nature and regulation of these particles differed between researches. Collagen fragments make up a significant portion of urine; of the 443 urinary peptides that have been identified, fragments of collagen I and III make up nearly 50% of the total (Coon J, 2008). Collagen-derived peptides' decreased excretion in urine has been hypothesized to be correlated to their increased extracellular matrix deposition, a sign of diabetic problems (Rossing K, 2008). One of the five main diagnostic criteria suggested by the Japanese Nephropathy Committee is increased urine collagen IV (Inomat S, 2005).

Alkhalaf et al. evaluated 65 DN biomarkers; utilizing a proteomic strategy and CE-MS. With 97% sensitivity and specificity, DN was assessed in a separate validation cohort of 70 participants. In a multicenter research cohort (n = 145), the sensitivity and specificity of a DN biomarker classifier created from 65 biomarkers were 93.8% and 91.4%, respectively. Only 34 of the 65 DN peptide biomarkers have been sequenced thus far. The majority of these were collagen fragments that were downregulated in the urine of DN patients, displaying

that alteration in the metabolism of collagen is correlated to renal damage. Observations include the regulation of alpha-1-antitrypsin fragments, an alpha-2-HS glycoprotein fragment, a beta-2-microglobulin fragment, serum albumin fragments, and a fragment of transthyretin. Interestingly, the amount of the same peptide with an additional change (oxidation) was higher in the urine of diabetic patients (Alkhalaf A,2010).

In a group of more than 600 patients and controls, Argile's et al. looked at the classification performance of the proteomic CKD273 based on 273 urinary peptides, which reliably permitted the precise detection of CKD. The CKD273 classifier distinguished CKD patients based on their renal function and provided information on the likelihood of suffering negative outcomes in a sizable independent cohort. The outcomes supported the utility of the CKD273 classifier in evaluating CKD and providing outcome information (Argile's A,2013).

Another study, using a blinded testing set, reported the identification of 2 biomarkers (ubiquitin and  $\beta_2$ -microglobulin) differentially expressed in the urine of patients with DN and nondiabetic patients with chronic kidney disease. The accuracy of these results was supported by renal biopsies and reached almost 90%. In both micro and macroalbuminuric diabetic patients, they identified a considerable excretion of 2MG and a preferential excretion of ubiquitin ribosomal fusion protein. These results can point to a potential role as novel biomarkers and a potential role in the pathophysiology of diabetic nephropathy (Papale M,2010).

The use of a panel with a combination of biomarkers rather than urinary albumin alone seems to be an interesting approach for early detection of DN, as these mechanisms contribute to the onset and outcomes of this disease. These mechanisms include glomerular damage (e.g., albumin), tubular damage (e.g., N-acetyl-(D)-glucosaminidase (NAG) and kidney injury molecule-1 (KIM-1), inflammation (e.g., TNF- $\alpha$ ) and oxidative stress e.g: 8-hydroxydeoxyguanosine (8-OHdG), (Moresco R,2013).

One of the latest approaches in biomarker discovery in DN relies on the assumption that, as podocytopenia and podocyturia progress, podocyte-specific proteins (such as synaptopodin, nephrin and podocin), should be lost and therefore detectable in the urine of patients with DN. It demonstrated that 100% of diabetic patients with microalbuminuria and macroalbuminuria have detectable nephronuria. Interestingly, small traces of nephrin were also found in half of the T2DM patients showing normoalbuminuria, suggesting the possibility that nephrin could serve as a fresh early DN biomarker (Jim B,2012).

## Conclusion

Finally, the use of urine proteomics as a tool for biomarker discovery is not limited to type 2 DN, recently; It was establish a precise link between the levels of a certain group of proteins and the state of diabetic evolution in a study concentrating on type 1 diabetes. The proteins with the lowest and second-highest excretion levels, respectively, in the urine of diabetic participants, when compared to those of healthy controls were Tamm-Horsfall urinary glycoprotein (THP) and zinc—2 glycoprotein (ZA2G). Additionally, the link between albuminuria and glycosylated hemoglobin and the amount of these proteins was very strong.

Currently, especially with the conclusion of the Human Genome Project, proteomics the systematic examination of proteins for their identity, amount, and function is acknowledged as an emerging field within modern sciences (Thongboonkerd V,2011). As automation rises, applications are made simpler, and costs fall, urinary proteomics is becoming more and more affordable. Promising novel indicators for the early detection of DN have discovered thanks to urinary proteomics (Zürbig P,2012)

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