

Analytical Methods and Clinical Applications in Renal Diseases for Human Urine Proteomics

Abstract

Imaging performed for unrelated therapeutic purposes is typically accidental in detecting renal cell carcinoma (RCC). Excellent outcomes for the surgical treatment of confined malignancies have been documented. With the widespread use of so-called “targeted medicines,” advanced RCC therapy has advanced significantly in recent years. One of the most difficult tasks in oncologic research is finding molecular markers in bodily fluids (such as sera and urine) that can be used for screening, diagnosis, follow-up, and monitoring drug-based therapy in RCC patients. Despite some encouraging findings concerning possible biomarkers in sera, there is little information now available about urine indicators for RCC. The most promising biomarkers found in the bodily fluids of RCC patients are reported in the review that follows.

Since urine is a useful source of proteins and peptides with a relatively stable composition and is simple to collect in large and repeated volumes using a noninvasive approach, it has drawn the interest of clinical proteomics experts in the past ten years. In this study, we go into great detail about the technical elements of urine proteomics, such as sample preparation, proteomic technologies, and their benefits and drawbacks. Acute kidney injury, focal segmental glomerulosclerosis, lupus nephritis, membranous nephropathy, and immunoglobulin A (IgA) nephropathy are only a few of the renal disorders for which new investigations using the urine proteome for biomarker development are reported. Additionally, a few of the readily accessible databases for urine proteomics are briefly discussed.

Keywords: urine proteomics • biomarkers • human serum albumin • immunoassays • mass spectrometry • fluorescence spectroscopy • chronic kidney disease • nephropathy

Introduction

Tissues and bodily fluids from patients, such as serum, plasma, urine, and saliva, are unquestionably useful sources for investigations to find new biomarkers for diagnostic purposes. The complexity of the protein composition is the first challenge in handling and analysing such samples. Urine is a more desirable resource for the examination of a wide range of disorders since it can be collected in a noninvasive and unrestricted manner and includes about 2000 proteins, making it a less complex sample than plasma, which has more than 10,000 core proteins. Additionally, compared to other bio fluids like plasma or serum, which are vulnerable to proteolytic degradation both before and after sampling, the protein content and

fragmentation of urine are rather stable [1].

Urine has been examined for determining the pathological process of both systemic and renal disorders since it is the result of blood filtration and also contains all proteins released from tubules and kidney-specific cells. While abundant serum proteins like albumin, immunoglobulin light chain, transferrin, vitamin D binding protein, myoglobin, and receptor-associated protein are reabsorbable in the proximal renal tubules primarily by endocytic receptors, megalin, and cubilin. Serum proteins are filtered at the glomeruli based on their sizes and charges. As a result, normal protein excretion is less than 150 mg per day, and typical protein

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content in donor urine is very low (less than 100 mg/L when urine output is 1.5 L/day). Compared to other body fluids like plasma, this is around a factor 1000 less. Proteinuria which is defined as the excretion of more than 150 mg/day of protein is a sign of glomerular or reabsorption failure [2].

Studies on urine proteomics take into account a number of obstacles, including low levels of total protein, high levels of salts and other substances that make it difficult to separate proteins, and rapid changes in urine composition throughout the course of the material collecting process. There have been significant attempts made to identify a method that is appropriate to examine the expression of particular urine marker proteins due to differences in sensitivity and accessibility of different proteomic approaches. In order to identify urinary excreted proteins and peptides in various stages of disease or therapy and to ascertain their quantities, functions, and interactions, urine proteomics is a potent platform. Proteomic methods can therefore reveal new treatment targets and disease mechanisms. This review focuses on the technical aspects of examining the urine proteome; it also examines the use of this platform in clinical settings and various urine proteome databases [3].

Discussion

Enrichment techniques may increase the likelihood of detecting disease-specific biomarkers because they are likely in the low abundant component of the proteome and are hidden by high abundant proteins. There are various procedures for enrichment, including the depletion of very abundant proteins utilising chromatographic-based enrichment techniques or commercial urine sample-specific kits. A number of highly abundant proteins, such as albumin, transferrin, haptoglobin, immunoglobulin G, immunoglobulin A, and alpha-1 antitrypsin, may be eliminated using antibody-based affinity depletion techniques, such as the Multiple Affinity Removal System (MARS), which can be combined with either 2DE or LC-MS analysis. In the peptide ligand library technique, enrichment of low abundant and depletion of high abundant proteins could be coupled. Using this technique, a variety of peptides immobilised on a solid-phase chromatographic matrix engage in particular

protein recognition interactions. Therefore, only proteins that have been captured are used in the analysis process, whereas proteins that have insufficient interactions are washed out and removed [4].

The concentration of peptides in the final eluent decreases as a result of peptide saturation for highly abundant proteins. Additionally, the specimen's protein dynamic range is reduced by concentrated low abundant proteins on their particular ligands. High beginning material amounts are necessary for this procedure to be successful; otherwise, the enriched profile would resemble the original profile without any enrichment. Strong cation exchange chromatography's (SCX) stationary phase's charge changes from positive to negative in aqueous solution, unlike anion exchange, and interacts with strongly basic analyses as a result. After washing the column with a pH-gradient solvent, the analyses are eluted. At higher pHs, the majority of the cationic proteins and peptides would elute from the column. As a result, this strategy has been presented as an effective way to enhance the phosphoproteome. Thongboonkerd et al. demonstrated the use of SCX to enrich the urine basic/cationic proteome. Current commercial protein depletion kits include the UPCK urine protein enrichment/concentration kit, which has been designed for proteomic research on urine samples. The literature suggests that utilising these depletion kits could boost the amount of low-abundance proteins found in urine by 2.5 times [5].

Protein separation can be accomplished using the reliable and popular two-dimensional gel electrophoresis technique. According to their isoelectric point and molecular mass, proteins are divided using this method, visualised, and semi-quantified by staining. The ability to identify relatively large molecules, calculate the pI and molecular weight of proteins, and look into posttranslational changes is all benefits of 2DE. Common 2DE technique drawbacks include the need for high protein concentrations, a lack of automation, the loss of extremely basic or acidic proteins (pH 3), the inability to detect large and small hydrophobic proteins ($M_r > 150$ kD), a lack of reproducibility, low throughput, and a limited dynamic range. Some technical advancement have allowed for some of these constraints. By using internal standards and pooling samples that have been labelled with various fluorescent dyes, the

development of two-dimensional difference gel electrophoresis (2D-DIGE) alleviated the problem of low throughput capacity and increased quantification accuracy and statistical confidence (Cy2, Cy3, and Cy5) [6].

In order to specifically investigate hydrophobic proteins, protein complexes, and protein-protein interactions on the gel matrix, the blue native approach was created. Proteins are divided using the blue native approach according to their two-dimensional molecular weights. Precast gels with multirun gel tanks, capable of running many electrophoretic gels at once, have been used to reduce the repeatability issue. Additionally, gels can be stained with various fluorescent dyes after being electrophoretically separated to improve the sensitivity of detecting spots. Desalting using chromatographic columns or filters is advised for studying urine proteins with 2DE in order to produce well-separated spots on the gel. Despite advancements in 2DE approach, researchers are less likely to use this method in their current investigations due to a lack of automation and laborious stages (it takes about 3 days to generate a 2DE pattern) [7].

Up to 40% of diabetic people might develop diabetic nephropathy (DN), a significant consequence of diabetes with a complicated aetiology. Microalbuminuria, which can lead to macroalbuminuria (>300 mg/24 h), and/or changes in serum creatinine levels, which indicate a reduction in glomerular filtration rate, are symptoms of DN. Loss of podocytes, thickening of the glomerular basement membrane, growth of mesangial cells, and tubule interstitial fibrosis are the histological manifestations of DN. The current method of diagnosing DN is biopsy, which is invasive and not advised in all situations of suspicion due to its negative effects. Biomarkers for urinary diagnostics, a promising noninvasive diagnostic alternative to biopsy, will soon be available in clinics [8].

Microalbuminuria (MA), the greatest predictor of DN currently available in the clinic, is crucial for the early identification of DN. However, numerous investigations have revealed that only a small proportion of MA patients proceed to proteinuria. Furthermore, many people with type 1 diabetes have already seen an early deterioration in renal function either intentionally or unintentionally concurrent with the onset of MA. Therefore, it appears

that MA may not be a sufficient early diagnostic biomarker of DN (although it may be useful for the detection of advanced DN) and that additional sensitive and specific markers must be discovered using proteomic approaches. Additionally, some of the proposed biomarkers for DN have been discovered in samples from patients, whose disease was identified by clinical criteria but not validated by biopsy, underscoring the need for more precise research on the identification of DN biomarkers. The study by Soldatos and Cooper was a carefully thought-out pioneering pilot study for the identification of early diagnostic markers by scanning the urine proteome of DN patients using SELDI-TOF [9].

With 93% sensitivity and 86% specificity, they were able to distinguish a 12-peak urine protein signature in type 2 diabetic patients from healthy controls. It's interesting to note that none of the individuals in their study had renal failure and were normalbunuric (albumin-to-creatinine ratio 30 mg/g). In a case-control study, Rossing found a correlation between levels of a group of proteins, particularly Tamm-Horse fall urinary glycoprotein (THP) and zinc—2 glycoprotein (ZA2G), and the progression of diabetes in type 1 diabetic patients. This was done using high-resolution two-dimensional gel electrophoresis separation and protein identifications by MALDI-TOF-MS and LC-MS/MS analysis. Nephrine has the potential to be a new early biomarker of DN because ELISA results showed that nephrinuria is detectable in all type 2 diabetes patients with macroalbuminuria and microalbuminuria. A longitudinal study used capillary electrophoresis-coupled mass spectrometry to analyse the urine proteome of DN patients. A potential early detection biomarker for macroalbuminuria that develops 3–5 years prior is altered collagen fragment content [10].

Conclusion

Other than accidental radiologic discovery, there is currently no diagnostic tool for early diagnosis of kidney cancer and no way to monitor recurrence or chemotherapy response. Biomarkers are molecules that can be easily quantified and used to track both normal and aberrant biologic performance. Unfortunately, there is currently no biomarker for the detection of kidney cancer. The currently available biomarkers seem to be

most useful as prognostic indicators, as adjuncts to diagnosis, and in patient follow-up.

Conflicts of Interest

None

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