Urinary Proteomics in Nephrotic Syndrome

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ABSTRACT

Nephrotic syndrome is the commonest glomerular disease. Typical symptoms could be proteinuria, low serum albumin and oedema. The mechanism of proteinuria in nephrotic syndrome is a defective glomerular filtration barrier. Renal biopsy is the gold standard for diagnosis of nephrotic syndrome currently which is invasive and based on histopathological features, therefore it seems to be necessary to search for noninvasive biomarkers to be used as the complementary tests in the diagnostics and prognostics of glomerular diseases, particularly when renal biopsy is limited or contraindicated. While a big proportion of urinary proteins originate from kidney tissue and these tissue specific proteins excrete more in kidney injury, therefore the identification of urinary proteins can further our understanding of renal dysfunction and renal disease including nephrotic syndrome. The interest of scientist to urinary proteomics is also growing for biomarker discovery. This review focuses on some types of nephrotic syndrome and proteomic studies applying urine specimen which have been reported.

Keywords: Nephrotic syndrome; urinary proteomics; noninvasive; renal biopsy.

INTRODUCTION

The nephrotic syndrome is defined by a urinary protein level exceeding 3.5 g per 1.73 m^2 of body-surface area per day [1]. It is also considered as the triad of proteinuria, low serum albumin and oedema [2]. The mechanism of proteinuria in nephrotic syndrome is
a defective glomerular filtration barrier. This barrier is impermeable to proteins in health but becomes permeable in nephrotic syndrome with the resultant loss of protein into the urine [2]. Furthermore, Nephrotic syndrome has been postulated to due to an abnormality in cell mediated immunity, which could affect the T-lymphocyte population, resulting in an increased production of lymphokines leading to increased glomerular filtration permeability to proteins [3]. Renal biopsy is the gold standard test for definitive diagnosis of nephrotic syndrome which vary in histological appearance and include: minimal change nephrotic syndrome (MCNS), focal segmental glomerulosclerosis (FSGS), diffuse mesangial sclerosis, mesangiocapillary glomerulonephritis and membranous nephropathy [2]. While there is no doubt that renal biopsy is the best and gold standard for the diagnosis of glomerular diseases including nephrotic syndrome, it is necessary to search for noninvasive biomarkers to be used as the complementary tests in the diagnostics and prognostics of glomerular diseases, particularly when renal biopsy is limited or contraindicated. Urinary biomarker discovery using proteomics tools is one of the most promising approaches for diagnosis and prognosis of kidney diseases. In this review, we imply some proteomics study using urine specimen for some types of nephrotic syndrome as well as a brief explanation regarding importance of urine as a valuable source of biomarkers.

Importance of Urine in proteomic studies:

Human urine is one of the valuable biological fluids and there are several reasons for considering urine the ideal medium for discovering biomarkers of diseases and being attractive protein source for proteomic studies: (1)

Collecting urine sample is noninvasive and it is easy to be obtained in large amounts, (2) proteins and peptides in urine are quite stable and less complex, and (3) the amount and composition of urinary proteome directly reflect changes in functions of the kidney and the urogenital tract (4) sampling can be repeated over time in the same individual for clinical surveillance [4-5].

Proteins in the urine are mainly composed of plasma proteins that pass through the glomerular filtration barrier as well as proteins secreted from the kidney and urinary tract [6]. More specifically, these proteins originate from glomerular filtration of plasma, excretion from epithelial cells in the urinary tract, sloughing of epithelial cells and casts, and formation of urinary exosomes [7].

The main drawback of using urine proteomics in clinical applications is that the urinary proteome changes greatly among different individuals, and among the same individual under different physiological conditions[6]. For instance, Urine specimens show a high degree of variability in volume; protein concentration (particularly in the case of kidney damage or dysfunction); total protein excreted pH(ranges from 4-8); as well as variability in urine components due to age, health, diet, or other factors; proteolysis while the urine is stored in the bladder; and degradation of collected urine samples upon storage [8]. Therefore, normalization of the amount or concentration of protein is critical for proteomics and protein biomarker experiments [9]. One example of normalization method is calculating the ratio of each protein's expression to the total protein in the sample [10].

The most important advantage of study the urinary proteome is the nature of noninvasiveness of collecting urine in comparison with biopsy. In addition, several factors related to the nature of the disease and patient status can directly impact on the effectiveness of renal biopsy [11] which they are granted using urinary proteome. For example, some conditions do not affect the kidney uniformly and the biopsy sample may not be representative of the disease involvement. A single urine specimen, sent from the ward or clinic to the laboratory for tests which provide sensitive and specific information on the disease process and prognosis, and also predict the response to treatment at an early stage of disease would provide the ideal [11].

As it was mentioned above, a big proportion of urinary proteins originate from kidney tissue, therefore the identification of urinary proteins can further our understanding of renal dysfunction and renal disease. Quantitative and qualitative analyses of urinary proteins have been used to study renal physiology and renal diseases [12-13].

To sum up, urinary proteomics provides a new direction for renal disease diagnosis, treatment, monitoring and prognosis.

Focal Segmental Glumerolosclerosis

Focal segmental glomerulosclerosis (FSGS) is a type of chronic nephropathy characterized by scattered sclerosis in some glomeruli (focal) in which only part of a glomerular capillary tuft is affected (segmental) [14-15]. Clinically, FSGS is classified as a type of nephrotic syndrome, with a massive proteinuria and sclerosis on glomeruli as the whole mark [16]. The most sensitive part of glomeruli which is affected by pathogenic factors in FSGS is podocytes (glomerular visceral epithelial cells) [14,17].

It is believed that effacement of foot process in podocytes and a defect in slit diagrams (Specialized tight junctions between podocytes) may cause leaking proteins into urine [18]. Damage to podocytes triggers apoptosis and also detachment of podocytes from the glomerular basement membrane [15,19]. The initial insult to the podocyte leads to further damage mediated by cytokine release, mechanical stress, and further loss of polarity, resulting in

sclerosis and scarring of the glomerulus [19, 20]. Abnormal metabolism of extracellular matrix (ECM) proteins, imbalance of sclerotic and anti-sclerotic factors, disturbed glomerular haemodynamics, and oxidative stress-induced damage and apoptosis of glomerular cells are some suggested mechanisms for FSGS [16].

Shui et.al in 2008 studied urinary proteome of mouse model using 2D gel-based proteomic approach to identify urinary proteins at pre-sclerotic and different sclerotic stages of FSGS [16].

Table 1. Human urinary biomarkers for focal segmental glumerulosclerosis**.**

Protein Name/ID	Technique	Cases
Albumin	2DE-MALDI-TOF-MS/ (SDS-PAGE)-IEF	FSGS-normal [22] FSGS(SSNS) [49]
Alpha-1-antitrypsin	2DE-MALDI-TOF-MS	FSGS-normal [22]
Transferrin	SDS-PAGE-IEF	FSGS (SSNS) [49]
IgG	SDS-PAGE-IEF	FSGS (SRNS) [49]
β_2-microglobulin	SDS-PAGE-IEF	FSGS (SRNS) [49]
lysozyme	SDS-PAGE-IEF	FSGS(SRNS) [49]
ICAM-1	ELISA	FSGS-normal [49]
TGF-β_1	ELISA	FSGS-MCD [50]
ß2-microglobulin	SELDI-TOF/MALDI-TOF-MS	SSNS-SRNS [51], [52]

SSNS: Steroid sensitive nephrotic syndrome, SRNS: Steroid resistant nephrotic syndrome, MCD: Minimal change disease.

Table 2. Human urinary biomarkers for IgA nephropathy**.**

Protein Name/ID	Technique	Cases
IL-8	ELISA	IgAN (advanced)-normal/IgAN(mild) [53]
Uromodulin	Magnetic beads-MALDI-TOF-MS	IgAN-normal [35]
signal-induced proliferation-associated protein 1	2DE-MALDI-TOF-MS	IgAN-normal [31]
insulin receptor precursor	2DE-MALDI-TOF-MS	IgAN-normal [31]
NADH- ubiquinone oxidoreductase	2DE-MALDI-TOF-MS	IgAN-normal [31]
HLA class I antigen	2DE-MALDI-TOF-MS	IgAN-normal [31]
BTB and kelch domaincontaining protein 3	2DE-MALDI-TOF-MS	IgAN-normal [31]
fructose-bisphosphate aldolase C	2DE-MALDI-TOF-MS	IgAN-normal [31]
clathrin heavy chain	2DE-MALDI-TOF-MS	IgAN-normal [31]
extracellular superoxide dismutase	2DE-MALDI-TOF-MS	IgAN-normal [31]
calponin	2DE-MALDI-TOF-MS	IgAN-normal [31]
GRB2-related adaptor protein 2	2DE-MALDI-TOF-MS	IgAN-normal [31]
-cGMP dependent phosphodiesterase	2DE-MALDI-TOF-MS	IgAN-normal [31]
Zinc finger protein 324	2DE-MALDI-TOF-MS	IgAN-normal [31]
Zinc finger protein 155	2DE-MALDI-TOF-MS	IgAN-normal [31]
Albumin	2D-DIGE-MS	IgAN-normal [32]
Transferrin	2D-DIGE-MS	IgAN-normal [32]
α_1-Antitrypsin	2D-DIGE-MS	IgAN-normal [32]
β-Globin	2D-DIGE-MS	IgAN-normal [32]
α_2-Globin	2D-DIGE-MS	IgAN-normal [32]
Carbonic anhydrase I	2D-DIGE-MS	IgAN-normal [32]
Cystatin C	2D-DIGE-MS	IgAN-normal [32]
Retinol-binding protein 4	2D-DIGE-MS	IgAN-normal [32]
α_2-Glycoprotein 1	2D-DIGE-MS	IgAN-normal [32]
α_1-Microglobulin	2D-DIGE-MS	IgAN-normal [32]

IgAN: IgA nephropathy

Table 3. Human urinary biomarkers for minimal change disease

Protein name/ID	Technique	Cases
IGFBP-1	Urine assay	MCD-FSGS [54]
IGFBP-2	Urine assay	MCD-FSGS [54]
IGFBP-3	Urine assay	MCD-FSGS [54]
Albumin	2DE-MALDI-TOF-MS	MCD-normal [22]
α-1 antitrypsin	2DE-MALDI-TOF-MS	MCD-normal [22]

MCD: Minimal change disease

Table 4. Human urinary biomarkers for Membranous nephropathy

Protein name/ID	Technique	Cases
Vascular endothelial growth factor	Sandwich enzyme immunoassay	MGN-normal [55]
Albumin	2DE-MALDI-TOF-MS	MGN-FSGS-DN-SLE [50]/ MGN-normal [22]
α-1 antitrypsin	2DE-MALDI-TOF-MS	MGN-FSGS-DN-SLE [50] /MGN-normal [22]
Complement factor B	2DE-MALDI-TOF-MS	MGN-FSGS-DN-SLE [56]
Haptoglobin	2DE-MALDI-TOF-MS	MGN-FSGS-DN-SLE [56]
Hemopexin	2DE-MALDI-TOF-MS	MGN-FSGS-DN-SLE [56]
Orosomucoid	2DE-MALDI-TOF-MS	MGN-FSGS-DN-SLE [56]
Retinol binding protein	2DE-MALDI-TOF-MS	MGN-FSGS-DN-SLE [56]
Transferrin	2DE-MALDI-TOF-MS	MGN-FSGS-DN-SLE [56]
Vitamin D binding protein	2DE-MALDI-TOF-MS	MGN-FSGS-DN-SLE [56]
Zinc α2 glycoprotein	2DE-MALDI-TOF-MS	MGN-FSGS-DN-SLE [56]

MGN: Membranous glomerulonephritis, DN: Diabetic nephropathy, SLE: Systemic lupus erythematosus

They identified 37 urinary proteins by MALDI-TOF MS showing characteristic patterns of dynamic changes along the disease course of FSGS which some of them were validated further by western blotting. Some of the Proteins with potential to affect glomerular sclerosis which were reported by this group include: collagen fragment, ECM protein 1, Bone morphogenetic protein (BMP)-7 and ADAM 32, Whereas Kallikrein, kininogen precursor, glutathione S-transferase, AIF-2, annexin A1 and E-cadherin were reported as proteins associated with haemodynamic problems, oxidative stress, apoptosis and epithelial cell damage.

Huang et al. in 2009 introduced Rab23 as a urinary biomarker for FSGS [21]. They also used 2D gel and MALDI-TOF MS. Candiano et al. studied urine protein patterns from ten children with steroid sensitive nephrotic syndrome (SSNS) and MCD, seven children with steroid resistant nephrotic syndrome (SRNS) and SRNS/FSGS and six adolescents and adults with memberanous nephropathy Using 2DE followed by MALDI-TOF MS they identified fragments of albumin and alpha-antitrypsin not seen in urine from healthy control [22]. Some of the important reported biomarkers for FSGS has been shown in table 1.

IgA nephropathy

However, IgA nephropathy itself is not consider as nephrotic syndrome, we would like to consider this glomerular disease here because it could lead to nephrotic syndrome eventually. Immunoglobulin A nephropathy (IgAN) or Berger's nephropathy [23-24] is the most common primary glomerulonephritis worldwide. IgAN is characterized by the predominant deposition of IgA in the glomerular mesangium. Diagnosis of IgA nephropathy is only possible by biopsy [25], which reveals the deposition of complexes containing polymeric IgA1 in the mesangium. Although progressive renal failure develops in most patients with IgAN, and 15–40% of patients with IgAN will eventually develop end-stage renal disease, the exact etiology of IgAN has yet to be clearly elucidated [26–28]. The clinical manifestations of IgAN range from asymptomatic microhematuria and proteinuria to massive edema with nephrotic syndrome, although acute renal failure is uncommon [29]. The pathogenic mechanism of

IgAN is still not completely understood. Up to our knowledge a few number of proteomics study have been done regarding IgA nephropathy, however they are more than proteomics experiments done in other glomerular diseases which will be implied in this review.

Haubitz M et al. in 2005 presented a specific urinary polypeptide pattern which could distinguish IgA nephropathy from healthy controls with a sensitivity of 100% and specificity of 90%, and from membranous nephropathy with a sensitivity of 77% and a specificity of 100%. Sequencing of three of the discriminatory polypeptides identified three different fragments of serum albumin [30]. In another study, changes in the expression of urinary proteins in patients with IgAN (n=13) were analyzed using 2-DE and comparison of their expression profiles to those of normal controls (n=12) [31]. Urinary proteins were identified by MALDI-TOF MS. Approximately 216 protein spots were detected as expressed differentially in IgAN. Among these, 82 spots were over-expressed, and 134 spots were under-expressed as compared to normal controls. A total of 84 differentially expressed spots, representing 59 different proteins, were finally identified in IgAN. Yokota and colleagues [32] employed 2D DIGE to define IgAN-specific urinary biomarkers. Comparing urinary proteomes between patients with IgAN (n=17) and healthy controls (n=10), they found increased levels of several urine proteins deriving from plasma, including: albumin and its degradation products (albumin fragments), transferrin, alpha-1-antitrypsin, and beta globin. Julian et al. [33] applied capillary electrophoresis coupled with MS (CE-MS) for diagnosis of renal disease characterized by IgA in glomerular immunodeposits. They defined the renal damage and IgAN patterns in a reference-set of 402 patients with various renal disorders and 207 healthy controls. Thongboonkerd and colleagues [34] applied microfluidic technology on a chip to the proteome profiling of human urine from 31 healthy controls, four patients with IgAN, and six patients with diabetic nephropathy. Comparison of urine proteome profile revealed nine different spectra in normal and diabetic nephropathy and three different spectra in diabetic nephropathy and IgAN. Wu et al. [35] used the combination of a magnetic bead separation system with MALDI-TOF-MS to analyze urinary peptides of IgAN patients (n=25). They reported a fragment of uromodulin (m/z1913,14) as a biomarker which distinguish IgAN patients from healthy controls. Table 2 shows some of the urinary biomarkers for IgAN obtained from human.

Minimal Change Disease

MCD is a glomerular disorder that typically leads to the sudden onset of nephrotic-range proteinuria. Affected individuals express additional features of the nephrotic syndrome (NS), including edema of the extremities and face, hypoalbuminemia, and hyperlipidemia [36]. MCD is the most common cause of NS in children, accounting for approximately 90% of cases in children aged <10 years and approximately 50% of cases in children >10 years [36,37]. In the majority of MCD cases, there is no identifiable etiology and the diagnosis is idiopathic or primary MCD. The relationship between MCD and primary FSGS is an active area of debate in nephrology. Podocyte injury with foot-process effacement is a central feature of both disorders, but segmental sclerosis of glomeruli is unique to FSGS [38]. MCD is a systemic disorder, possibly caused by a circulating factor in the serum, rather than a pathological entity restricted to the kidney [39]. Although the pathogenesis of minimal change glomerulopathy remains unclear, this disorder is most likely a consequence of T lymphocyte abnormalities. T cells apparently produce a product, most likely a lymphokine, that increases glomerular permeability to protein. When the glomerular permeability factor is removed from the kidney, it functions normally. Theses factor may have specificity for glomerular epithelial cells that results in loss of the charge-selective barrier of the glomerular basement membrane [40].

Cutler et al. [41] performed 2-DE analysis to evaluate changes in urinary proteome profile of puromycin aminonucleoside (PAN) induced nephrotoxic rats which showed clinical features resembling human minimal change nephropathy compared to the controls (n =5 each). At the baseline, rat urinary proteins were mostly present at low-molecular weight range with two major

abundant proteins, a2u-globulin and glial fibrillary acidic protein. An administration of PAN caused a shift of spot pattern of urinary proteins toward the higher molecular weight range.These shifted proteins included albumin, transferrin, and vitamin D-binding protein. These changes were abolished after 672 h of a single PAN administration.

Up to our knowledge there is no proteomic study on human urine specimen for minimal change disease specifically. However, there are a few urinary proteomic studies on comparison several types of glomerular diseases including minimal change disease in a single study. For instance, Weissinger et al. compared urine samples from patients with MCD, FSGS, MGN and healthy controls to identify peptide expression patterns. The classification accuracy was 71.4% for MCD and FSGS and 92.9% for MGN [42]. Table 3 shows some urinary biomarkers for MCD.

Membranous Nephropathy

Membranous nephropathy (MN) remains the most common cause of nephrotic syndrome in adults age (40-60 years), and it is a leading cause of renal failure within the primary glomerulonephritis group.[43,44]. Membranous glomerulopathy occurs as an idiopathic (primary) or secondary disease. Secondary membranous glomerulopathy is caused by autoimmune diseases (e.g., lupus erythematosus, autoimmune thyroiditis), infection (e.g., hepatitis B, hepatitis C), drugs (e.g., penicillamine, gold), and malignancies (e.g., colon cancer, lung cancer) [40]. It is characterized histologically by the uniform thickening of the glomerular capillary wall on light microscopy [45]. This thickening is associated with subepithelial immune complex deposits that appear as granular deposits of immunoglobulin (Ig) G on immunofluorescence and as electron-dense deposits on electron microscopy. Symptoms could be proteinuria, microscopic hematuria and sometimes Hypertension [45-46].

Ngai et al. [47] performed serial analysis of urinary proteome profile of a rat model of passive Heymann nephritis (PHN), which resembles human membranous nephropathy. They used 2-DE technique and also MALDI-TOF and find 37 proteins which significantly differed in 6 time points. Most of these altered proteins have functional significance in signaling pathways, glomerular trafficking, and controlling the glomerular permeability. The other group also used 2D-DIGE and MALDI-TOF-MS to find differential proteins between urine proteome of PHN induced rat and normal cases [48].

Among the identified proteins, the markedly increased level of urine haptoglobin (approximately ten-fold increase) was clearly confirmed by Western blot analysis and ELISA. Table 4 shows some human urinary biomarkers reported for MN.

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