African Journal of Biotechnology Vol. 7 (18), pp. 3166-3172, 17 September, 2008
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB08.287
ISSN 1684–5315 © 2008 Academic Journals

Review

Clinical and diagnostic importance of proteinuria: A review

Oni M. O.[1] and Oguntibeju O. O.[2]*

[1]Department of Clinical Biochemistry, Faculty of Applied Sciences, University of the West of England, Bristol, England.
[2]Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa.

Accepted 18 June, 2008

A continual function of the kidney is essential to good health playing active roles in urine formation. Under normal physiological state urine is expected to be protein free. The production of protein free urine is exclusively carried out by the kidney nephrons. Nephrons are structured to perform an important role of filtration and reabsorption. Therefore, a defective or diseased kidney is associated with defective reabsorption mechanisms and an indication of injured nephrons. The appearance of proteins in urine (proteinuria) is a strong indicator of kidney disease. The assessment of proteinuria is clinically and diagnostically an important index in renal function generally and particularly that of nephrons. However, pathological proteinuria may be due to various factors or diseases. It is has been shown that diabetis mellitus, cardiovascular disease and hypertension could provoke secondary kidney problems. Proteinuria is also significant in some non-pathological cases such as pregnancy and static proteinuria found among military men. Various methods are used for the screening and detection of pathological and non-pathological proteinuria; the methods have their individual merit and demerits. Researchers had made significant contributions in improving the traditional techniques used in detecting and estimating urine protein. Current research in proteomics and genomics science with the development of new techniques in chromatography. (2D-gel chromatography) had produced interesting and promising results in discovery of other classes of urine proteins that could be used as biomarkers of renal disease. This review examines the clinical and diagnostic importance of proteinuria and available techniques. It is hoped that this paper would throw more light into this important but taken-for-granted diagnostic tool in laboratory medicine.

Key words: Glomerular filtration, clinical, diagnostic, proteinuria, nephrons.

INTRODUCTION

According to Kanwar and colleagues (Kanwar et al., 1984), urine is a modified ultrafiltrate of plasma free of protein (under normal physiological state) produced via glomerular capillary wall. Glomerulus is structured with millions of capillaries on its walls. The mechanisms of the ultrafiltration involve the magnitude and direction of the hydraulic and colloid osmotic pressures across the capillary walls (Anderson et al., 2000). The use of tracer macromolecules of well defined size and shape such as dextrans, ficoll, and proteins have been widely used to describe the glomerular capillary wall permeability. The size and charge selectivity of the capillary wall, in addition to the charge, shape, deformability and size of the macromolecules play major roles in the clearance processes (Deen et al., 2001). Glomerular diseases are characterized by defects in both size and charge selectivity of the glomerular capillary wall and result in the presence of proteins in urine, termed “glomerular proteinuria” (Anderson et al., 2000).

Structure of the kidney

The kidneys are situated on both sides of the posterior part of the abdomen, behind the peritoneum. Each kidney

*Corresponding author. E-mail: bejufemi@yahoo.co.uk. Tel: +27 21 959 6917.

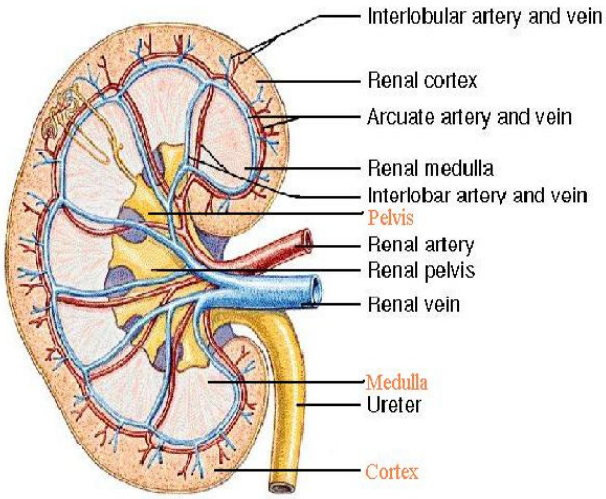

Figure 1. Showing cross section of human kidney, adapted from www.ivy-rose.co.uk.

(Figure 1) is about 11 - 12 cm long and weighs about 150 g. The kidneys contain about two millions glomerulae (Kriz et al., 2000; Hoy et al., 2003). Kidney nephrons are the functional units of the kidneys (Figure 2). There are typically over 10,000 kidney nephrons in each of the two kidneys in the body. The glomerulus is a lobulated network of convoluted capillary blood vessels surrounded by the Bowman's capsule. The total length of the capillaries, in a single glomerulus is approximately 9.5 mm with 9 - 12 µm in diameter resulting in an overall capillary length of 19 km, and a glomerular surface area of approximately 1 m^2 in the kidneys (Rostgaard and Qvortrup, 2002).

Glomerular filtration

The main activity of the glomerulus is to produce an ultrafiltrate from the blood using the glomerular capillary wall as a filter (Figure 3). The glomerular filtration process exhibits distinguished mechanisms from the transcapillary exchange process as in other organs. Glomerular capillary wall usually excludes plasma proteins of the size of albumin and larger molecules from the filtrate. In addition, the glomeruli have an extraordinary high permeability-surface area product (PS) to water and small solutes and also a very high capillary filtration capacity. Fluid traffic across the glomerulus is similar to the conditions in other capillaries, controlled by the Starling forces, (that is, the differences between the effective hydrostatic pressure gradient and the effective oncotic pressure gradient) (Chang et al., 1975).

$$GFR = LpS \times (\Delta P - \Delta \Pi)$$

Where, Lp represents the hydraulic conductivity of the glomerular capillary wall, and S is the surface area available for filtration. ΔP is the hydrostatic pressure in the glomerular capillaries minus the hydrostatic pressure in the Bowman's space, and $\Delta \Pi$ the effective oncotic pressure in the glomerular capillaries minus that in the Bowman's space. Glomerulus filtration rate (GFR) can be measured clinically using molecules that are metabolized freely and filtered across the glomerulus and that are not bound to plasma proteins nor are absorbed or secreted by the tubules, for example, inulin or Cr EDTA. Normal GFR in females is stated as 95 ± 20 ml/min and 120 ± 25 ml/min for males (Toto, 1995).

The size and selective function of the Glomerular capillary wall has been broadly studied using transglomerular filtration of tracer macromolecules. It was noted that the ratio of filtrate-to-plasma concentration and macromolecule such as albumin in reference to solute such as Cr EDTA, found in Bowman's space in almost equal amount as in plasma water. The ratio is referred to as "fractional clearance" or "sieving coefficient" (θ) of the transported macromolecule through the glomerular capillary wall (Bohrer et al., 1978).

The mechanism of glomerular capillary wall permeability

The glomerular capillary wall consists of the glomerular basement membrane (GBM), an endothelial cell layer and an epithelial cell layer. These cell layers are covered with a negatively charged surface coat (10 - 60 nm thick), referred to as the glycocalyx. In addition, a much larger exclusion area extending from the endothelial surface for anionic macromolecules, possibly composed of glycosyated macromolecules and adsorbed plasma proteins, has been described represented as "endothelial surface layer" (ESL). The fenestrae (space) between the endothelium cells are 50 - 60 nm in diameter, and also appear to be filled with plugs of glycocalyx or ESL up to 90 nm in height. They are thought to provide the glomerular capillary wall with size and, most importantly, with charge-selectivity.

The ultra filtration of macromolecules such as proteins is dependent upon their size, charge and structure, as well as the pore size and electrostatic properties of the glomerular filtration barrier. In addition, renal homodynamic factors may influence the clearance of macromolecules. Despite the extremely low resistance to the flux of water, the human glomerular filter very efficiently restricts the passage of macromolecules from blood into Bowman's space. The passage of low molecular weight (LMW) proteins, for example, proteins smaller than 30 kDa/molecular weight (MW) and with a radius smaller than 25 Å, usually have limited restrictions in normal individuals (Maack et al., 1979). The estimated albumin concentration in normal glomerular ultrafiltrate is only about 20 mg/L compared to approximately 40000 mg/L Concentration of the protein in human plasma. Thus, the glomerular sieving coefficient of albumin is 5 - 6 x 10^{-4}

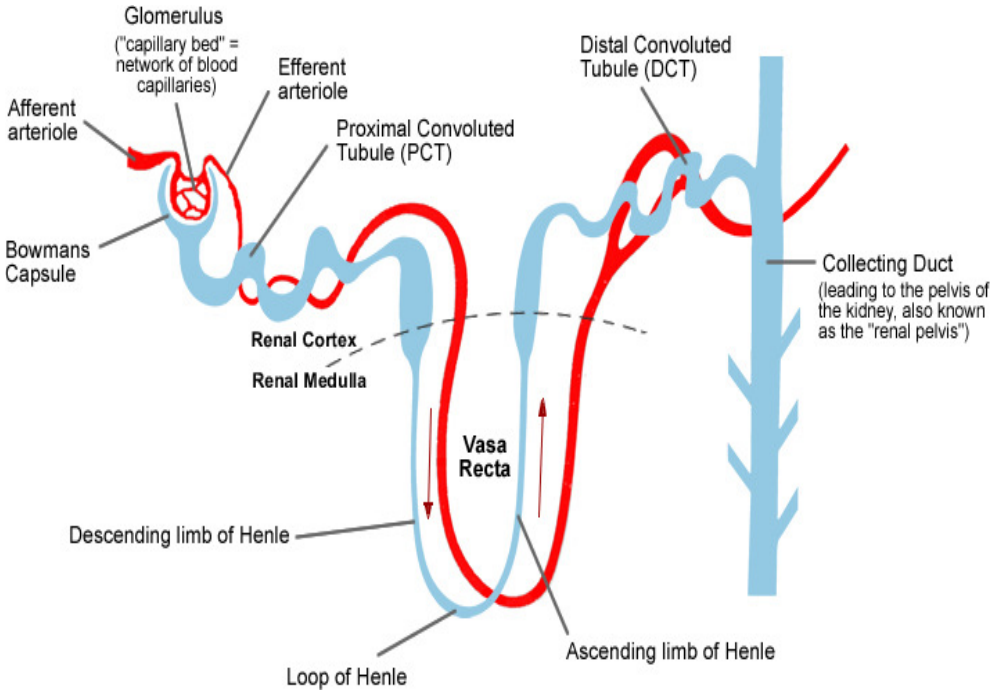

Figure 2. Showing the labeled diagram of human nephron adapted from www.ivyrose.co.uk.

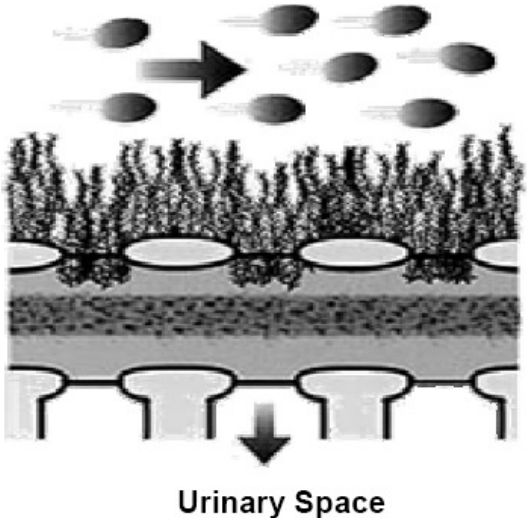

Figure 3. Diagram of the glomerular capillary showing the luminal surface coat lining the endothelium, and filling out of the fenestrae, the ultrafiltrate passes through the downstream layers of the Bowman's capsule adapted from www.ivyrose.co.uk.

(Landwehr et al., 1977). The opinion that the glomerular filter is a highly size- and charge-selective barrier has been critised recently by researchers. They noticed that sieving coefficient (θ) for albumin was nearly a 100-fold higher than the earlier reported values and argued that there are limited evidences for charge-selectivity of the glomerular filter (Russo et al., 2002).

Accepting their explanations then means minimum of 600 g of albumin would pass the human glomerular filter in twenty-four hours. As a result, it was suggested a non-degradative 'retrieval pathway' to account for the reabsorption to plasma of almost the total filtered load of albumin. Further, a considerable fraction of urinary proteins was found degraded and eliminated in the urine as protein fragments. Studies showed that a reduced protein 'retrieval' to plasma, or reduced protein degradation, would be mainly accountable for the increase urinary protein excretion appearing in most proteinuria disorders. Though, there is convincing evidence that the conventional view is still the most acceptable (Ohlson et al., 2002). The most widely used description of macromolecular transport across the glomerular capillary wall indicates that the glomerular filter is perforated by pores having either a continuous normal distribution of radii, or paired discrete populations of pores. This theoretical picture of glomerular capillary wall does not explain the ultra-structural analyses but on a hydrodynamic theory of obstructed solute traffic through water permeable membrane, as explained by Pappenheimer in 1951 (Pappenheimer et al., 1951). The two-pore or paired pores theory of capillary permeability adequately explains the fractional clearance often used in scientific experiments and in clinical studies (Lund et al., 2003). In the "two pore with a shunt model" the large majority of pores are "small pores". The small pores exhibit a radius of ≈29Å with negative charges, stiff, spherical proteins, and a radius of 37 - 38Å having uncharged macromolecules. The second pore population consists of a very

small number of “large pores” of radius 90 - 115Å (Tencer et al., 1998). The small pores are basically impermeable to macromolecules of equal size of albumin or larger. Such molecules are normally transported by convection across the large pores. In addition to the two pores, the glomerular capillary wall may display “shunts”, which are very sporadic physiological “membrane defects”, large enough to allow the transport of very large proteins and even red blood cells. Proteins such as IgM (radius 120Å) are able to pass through the glomerular capillary wall only through these shunts. It is conceived that a repairing apparatus normally seals these shunts, and an increased transport of IgM indicates unsealing of the shunts and/or increased density of these defects in the glomerular capillary (Schurek et al., 1992).

PATHOGENESIS OF PROTEINURIA

There are three major paths. The first is physiological mechanisms that produce proteinuria. These are: glomerular, tubular and overflow glomerular dysfunction. Overflow glomerular dysfunction is the most common and usually results in urinary protein excretion of more than 2 g/day. The second: the epithelial cells of proximal tubules are actively involved in the catabolism of a number of peptides and plasma proteins which is believed to lead to degradation within the lysosomes. Different proteolytic enzymes activities are known to take place in the kidney; however, there are no sufficient explanations to the exact lysosomal enzymes involved in the increased protein catabolism in the proximal tubule. Hyperactivity of the lysosomal pathway has being implicated in renal disease associated with raised excretion of urinary proteins. Many glomerular malfunctions alter the permeability of the glomerular basement membrane, resulting in pathological albuminuria and immunoglobulinurias (Abuelo, 1983). Excessive excretion of proteins in the urine is the hallmark of clinical glomerular diseases. Proteinuria is a signal of defective glomerular capillary wall and in tubular protein absorption. It can be an effective assessment of the overall severity of the glomerular and tubulointerstitial damage, and consequently, a good prediction of glomerular diseases Christensen and Birn, 2001). The reabsorption of proteins in pre-tubular urine is usually carried out by the receptor-mediated endocytosis in the proximal convoluted tubules (Christensen and Birn, 2001). Megalin and cubilin are the two major receptors essential for normal tubular reabsorption of filtered proteins.

The lysosomes hydrolyzed the absorbed proteins to amino acids, these amino acids passed through basement membrane and re circulated back to the system (Leheste et al., 1999). It is believed that about 90 – 95% of the filtered albumins are reabsorbed in the proximal tubules, but there is total reabsorption of low molecular weight Proteins. In tubulointerstitial injury there is an impairment of proximal tubular uptake of filtered proteins, this leads to increased urinary excretion of low molecular weight proteins, the distinguishing feature of tubular proteinuria (Norden et al., 2000).

Mesangial and tubulointerstitial damage resulting to renal injury has being attributed to proteinuria (Iseki et al., 2003). Actually, proteinuria is the major determinant of progressive renal failure and the most broadly anticipated source of tubular injury in proteinuric glomerular disorder is the extensive tubular uptake of filtered plasma proteins, including growth factors and complement factors, cytokines and protein bound substances, such as fatty acids, bound with the filtered albumin. These factors may stimulate tubular generation of vasoactive and inflammatory cytokines, leading to spreading of inflammatory cells to the interstitial (Eddy, 2004). In cases of predominantly selective proteinuria, where albumin is almost completely lost in urine, the occurrence of tubulointerstitial injury is uncommon, and the kidney activities remain intact (Iseki et al., 2003). However, in non-selective proteinuria, there is progressive tubulointerstitial injury, which in most case progressed to renal insufficiency (Branten et al., 2001).

Classification of proteinuria

Identification of protein in urine and its association with renal disease date back to the days of Hippocrates (Woo et al., 2000; Beetham et al., 1993). Proteinuria is acceptably defined as urinary protein excretion that exceeds 150 mg/day. Urinary protein excretion in healthy individuals varies significantly and may reach suspected pathological levels under several factors. Most dipstick tests (for instance, Albustin, Multistix) that are positive for protein are a result of benign proteinuria, which is related to morbidity or mortality. Common causes of benign proteinuria include dehydration, emotional stress fever and heat injury, inflammatory process while others include intense activity, acute illnesses and orthostatic (postural) disorders. Almost one fifth of normally excreted protein is a low-molecular-weight type such as Light chain (molecular weight about 20,000 Daltons), 40 percent is high-molecular-weight albumin (about 65,000 Daltons) and 40 percent is made up of Tamm-Horsfall mucoproteins secreted by the distal tubule. (Beetham et al., 1993; Hsu and Couser, 2003).

In Glomerular associated proteinuria there is usually significant increased glomerular capillary permeability to protein that may be due to either primary or secondary glomerulopathy. Tubular proteinuria occurs when tubulointerstitial disease prevents the proximal tubule from reabsorbing low-molecular-weight proteins (part of the normal glomerular ultrafiltrate). When a patient developed tubular disease, usually less than 2 g of protein is excreted in 24 h. Tubular diseases include hypertensive nephrosclerosis and tubulointerstitial nephropathy caused by nonsteroidal anti-inflammatory drugs. In overflow proteinuria, low-molecular-weight proteins overwhelm the ability of the proximal tubules to reabsorb filtered proteins. Most often, this is a result of the light chain over-

production that occurs in multiple myeloma. The resultant light-chain Immunoglobulin fragments (Bence-Jones proteins) produce a monoclonal spike in the urine electrophoresis' pattern. (Stone, 1989; Longo et al., 1998)

Albumin

This is the largest circulating plasma protein, (69 kDa MW, serum concentration (38 – 40 g/L) and functions in maintenance of oncotic pressure, acid-base changes buffering, and transportation of various bioactive substances such as fatty acids, steroid hormones and vitamins (Pratt and Kaplan, 2003). Albumin is mainly produced in the liver and the endothelial cells are responsible for its breakdown. The serum albumin concentration is a good indicator of its synthesis and breakdown, its quantity in circulation, the accessibility of amino acid precursors and a predictor of the amount of albumin loss into urine, intestinal lumen and from the skin (Longo, et al., 1998; Pratt and Kaplan, 2003)

Immunoglobulins (antibodies)

Are complex groups of proteins that are functionally and structurally related that protect the body from invasion by pathogenic microorganisms and their toxic products. The basic structure of the immunoglobulin molecule consist of a monomer that contains four polypeptide chains, two heavy chains (each of 50 kDa MW) and two light chains (kappa or lambda, each of 20 kDa MW) linked by disulfide bonds (Ballantyne et al., 2000). There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM antibodies.

Immunoglobulin G (IgG)

Is a monomeric molecule of 150 kDa, which predominates in secondary or memory immune response against infectious organisms. It is found on surface of memory B cells and predominantly in the blood. It constitutes 75% of serum immunoglobulin. Normal serum concentration of IgG is 5 – 15 g/l. There are four subclasses of IgG, differing in the number of disulfide bonds and in the length of the hinge region. IgG1 has the highest concentration in human serum (3 - 10 g/l), followed in order by IgG2 (1 - 3.5 g/l), IgG3 (0.3 - 1 g/l), and IgG4 (0.2 - 0.5 g/l)" (Bonilla, 2003). Since IgG2 is neutral and IgG4 is negatively charged, a low value of urine IgG2/IgG4 ratio reflects loss of charge selectivity of the glomerular capillary wall.

Immunoglobulin M (IgM)

Is the third most common serum IgM with serum concentration of 0.5 - 4 g/l. IgM is composed of five complete IgM units linked by disulfide bonds to form a pentamer with a molecular weight of 950 kDa (Grubb et al., 1983). IgM is the first Immunoglobulin to be made by the foetus and the first antibody made by a virgin B cells when it is stimulated by antigen. As a result of its pentameric structure, IgM is a good agglutinating and complement fixing Immunoglobulin, very efficient in clumping and lysis of microorganisms.

Protein (Human complex) HC

This is also known, as α1-microglobulin-is a human complex forming glycoprotein, heterogeneous in charge, and was first described by Tejler and Grubb (1992). It is composed of a single polypeptide chain and its physiological function is unknown. Bonds between human complex protein and IgA and between protein HC and albumin are present in most human biological fluids but they are not present in normal urine and rarely in pathological urine (Tejler et al., 1992). Only the free form of protein HC is filtered through the glomerular capillary wall and thus, found in normal urine. Protein HC is relatively stable in urine and is also stable at low urinary pH values. The high sensitivity of increased urinary excretion of protein HC makes the determination of the urinary excretion of protein HC an ideal biomarker for demonstration of proximal tubular disorders (Zhongxin et al., 2005).

Proteinuria as a biomarker of kidney disease

The presence of filtered high molecular and tubular proteins in urine has been the conventional biomarker indicating impairment and predicting sequence in chronic renal disease. The predictive usefulness of proteinuria was earlier discovered in known cases of diabetic type 1 with significant presence of albumin in their urine and subsequently developed nephropathy secondary to type 1 diabetics. (Eknoyan et al., 2003; Hunsicker et al., 1997). Proteinuria is regarded as the most acceptable predictive variable in prognosis of nondiabetic chronic renal disease progression (Eknoyan et al., 2003). Proper interpretation of proteinuria required to be in line with the clinical context and may be based on other factors. For instance, there had been reported cases of complete resolution of miroalbuminuria among diabetic subjects and were found to be normoalbuminuria with 48 months of follow-up (Gorman et al., 1999). Unfortunately, these proteins effectiveness as biomarkers suffered from lack of specificity and standardization of the techniques.

Methods of assessing proteinuria

Accurate and rapid detection and quantitation of urinary

proteins are important not only in prognosis and diagnosis of kidney diseases but also in the management of these diseases and other diseases that induced nephropathy such as diabetic mellitus, hypertension. Assessing proteinuria is considerably helpful in the management of hypertensive disorders in pregnancy and other diseases (Hunsicker et al., 1997).

Dipstick urinalysis

Dipstick urinalysis, which is commonly done as the screening test for proteinuria, is very accessible and mostly used in point of care testing for the screening of hypertensive pregnant women and suspected nephrotic syndrome cases in children. Though very popular and easy to use, the alarming rate of false negative and exaggerated false positive values, are the shortcomings of this technique in detecting proteinuria. The factors that may affect the preparatory reagents (dipstick) used in the dipstick include storage, expiring date, contaminations, handling and these factors may account for the inadequacies associated with its use in urinalysis.

Automated urinalysis

The introduction of automated urinalysis gave birth to an era of more accurate detection of urinary proteins. The use of automated analyzers had carefully removed the visual variations encountered in dipstick methods. Though studies showed that automated urinalysis improved the predictive power of urinalysis even at low concentration of about 0.3 g/L equivalent of 1+ in dipstick (Hunsicker et al., 1997). Further studies on the comparability of automated urine analyzer showed that its readings were in line with results obtained in visual technique in urinary protein analysis in children with variety of glomerular diseases. The technicality and the skill needed in automated method makes it cumbersome for screening of patients hence may not be ideal for point of care testing.

Electrophoresis Technique

Protein electrophoresis is usually performed using agarose media, this convectional method is widely used to differentiate between normal renal function, glomerular proteinuria, and tubular proteinuria. Increased glomerular permeability results in higher concentrations of high molecular weight proteins in the glomerular filtrate. Reduced tubular reabsorptive capacity usually lead to noticeable increase in urinary excretion of low molecular weight proteins its limitation lies on low sensitivity. However, it gives optimal result when looking for a free monoclonal light chain (Bence Jones protein) is a concentrated urine specimen.

NEW INNOVATION TECHNIQUES AND DISCOVERY OF OTHER PROTEINS OF INTEREST IN URINE

Progressive successes recorded in technologies have drastically enhanced the rate and accuracy of testing and estimating proteins in biological fluids. The advent of proteomic technology has produced a number of reliable urinary proteins that are specific biomarkers for kidney diseases. Proteomics is the systematic analysis of expressions of proteins in tissues, by isolation, separation, identification and functional grouping of proteins (Anderson et al., 2000). Major proteins that are found in urine sample through proteomic technologies are neutrophil gelatinase- associated lipocalin (NGAL)–a 25 kda covalently bound to gelatinase from neutrophils, and interleukins -18 (IL-18). Interleukins is a proinflammatory cytokine stimulated and cleaved in the proximal tubule and later appear in urine. Its level was found to be significantly raised in acute renal injury (Parikh et al., 2004).

Besides, a transmembrane molecule commonly referred to as kidney injury molecule (KIM) has been reported to be present in the urine and useful in differential diagnosis of acute kidney injury, as urinary KIM remains normal in acute renal dysfunction but significantly increased in chronic renal disease (Liangos et al., 2006). Though (NGAL) is very sensitive to acute renal damage, KIM-1 had being found to be very specific to acute renal injury (Han et al., 2006). The analysis of urinary proteins can be carried out in a number of techniques; these include immunoblotting, gel electrophoresis, mass spectrometry and enzymatic or metabolic assays. These methods independently provide different types of information and each of them has its own qualities and shortcomings.

The current electrophoresis technique used in urinary proteins analysis involves the use of 2-dimensional (2-D) gel electrophoresis. This method reflects similar protein profiles for glomerular toxicity to other techniques but with more in depth knowledge of the nature and development of the related glomerular damage proteinuria. Further, 2-dimensional (2-D) gel electrophoresis had been reportedly useful for early detection of proteinuria; it therefore became an important comparatively early biomarker and superior to the gross urinary protein determination procedure (Antonio et al., 2005). Unfortunately, the use of 2-dimensional (2-D) gel electrophoresis in proteomic analysis is limited due to its lack of sensitivity.

Future development

The emergent proteomic and gene expression should be highly exploited to detect other urinary proteins of interest associated with renal pathology with the singular aim of developing an increased use of non-invasive technologies without compromising the sensitivity and specificity of the methods and the tests. Also, it would be appropriate to conduct cheaper; higher dependable measurement on variables that are of more interest in proteinuria eva-

luation process using nanotechnology to develop devices suitable in point of care testing that would be beneficial to the patients and be cost-effective.

REFERENCES

Abuelo JG (1983). Proteinuria: diagnostic principles and procedures. Ann Int. Med. 98: 186-191.

Anderson NL, Matheson AD, Steiner S (2000). Proteomics: Applications in basic and applied biology, Curr. Opin. Biotechnol. 11: 408-412.

Anderson S, Tank JE, Brenner BM (2000). Renal and Systemic Manifestations of Glomerular Disease In: Brenner BM, Levine SA, editors. Brenner & Rector's The Kidney Sixth ed. Philadelphia: W. B. Saunders Company., pp. 1871-1900.

Antonio C, Jesús H, Xosé ML, Carlos J (2005). A comparative study of three kidney biomarker tests in autosomal-dominant polycystic kidney disease. Kidney Int. 68: 948-954.

Ballantyne J, Mandle R, Bing DH (2000). Immunoglobulins: Polyclonal and Monoclonal Antibodies. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE editors. HEMATOLOGY: Basic Principles and Practice. 3rd edition. New York: Churchill Livingstone; pp. 640-650.

Beetham R, Cattell WR (1993). Proteinuria: pathophysiology, significance and recommendation for measurement in clinical practice. Ann. Clin. Biochem. 30(pt5): 425-434.

Bohrer MP, Baylis C, Himes HD, Glassock RJ, Robertson CR, Brenner BM (1978). Permselectivity of the glomerular capillary wall. Facilitated filtration of circulating polycations J. Clin. Invest. 61: 72-78.

Bonilla FA (2003). Structure of immunoglobulins In 11.3 edition Up-To-Date.

Branten AJ, van den Born J, Jansen JL, Assmann KJ, Wetzels JF (2001). Familial nephropathy differing from minimal change nephropathy and focal glomerulosclerosis Kidney Int. 59(2): 693-701.

Chang RS, Ueki IF, Troy JL, Deen WM, Robertson CR, Brenner BM (1975). Permselectivity of the glomerular capillary wall to macromolecules II. Experimental studies in rats using neutral dextran. Biophys. J. 15: 887-906.

Christensen EI, Birn H (2001). Megalin and cubilin: synergistic endocytic receptors in renal proximal tubule. Am. J. Physiol., Renal Physiol. 280(4): F562-573.

Deen WM, Lazzara MJ, Myers BD (2001). Structural determinants of glomerular permeability. Am. J. Physiol. Renal Physiol. 281(4): F579-596.

Eddy AA (2004). Proteinuria and interstitial injury, J. Nephrol. Dial Transplant; 19(2): 277-281.

Eknoyan G, Hostetter T, Bakris GL, Hebert L, Levey AS, Parving HH, Steffes MW, Toto R (2003). Proteinuria and other markers of chronic kidney disease: a position statement of the National Kidney Foundation (NKF) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Am. J. Kidney Discov. 42: 617-622

Gorman D, Sochett E, Daneman D (1999). The natural history of microalbuminuria in adolescents with type 1 diabetes J. Pediatr. 134: 333-337.

Grubb AO, Lopez C, Tejler L, Mendez E (1983). Isolation of human complex-forming glycoprotein, heterogeneous in charge (protein HC), and its IgA complex from plasma Physiochemical and immunochemical properties, normal plasma concentration J. Biol. Chem. 258(23): 14698-14707.

Han WK, Waikar SS, Johson A, Curhan GC, Devarajan P, Bonve JV (2006). Urinary biomakers for early detection of acute kidney injury. J. Am. Soc. Nephrol. 17: 403.

Hoy WE, Douglas-Denton RN, Hughson MD, Cass A, Johnson K, Bertram JF (2003). A stereological study of glomerular number and volume: Preliminary findings in a multiracial study of kidneys at autopsy. Kidney Int. 63(s83): s31-s37.

Hsu SI, Couser WG (2003). Chronic progression of tubulointerstitial damage in proteinuric renal disease is mediated by complement activation: a therapeutic role for complement inhibitors? J. Am. Society Nephrol. 14(7 Suppl2): S186-191.

Hunsicker LG, Adler S, Caggiula A, England BK, Greene T, Kusek JW, Rogers NL, Teschan PE (1997). Predictors of the progression of renal disease in the modification of diet in renal disease study Kidney Int. 51:1908-1919.

Iseki K, Ikemiya Y, Iseki C, Takishita S (2003). Proteinuria and the risk of developing end-stage renal disease Kidney Int. 63(4): 1468-1474.

Kriz W, Elgar M (2000). Renal Anatomy. In: Johnson RJ, Feehally J, editors. Comprehensive Clinical Nephrology First edition London: Harcourt Publisher Limited; p. 1.1-1.10.

Landwehr DM, Carvalho JS, Oken DE (1977). Micropuncture studies of the filtration and absorption of albumin by nephrotic rats. Kidney Int. 11(1): 9-17.

Leheste JR, Rolinski B, Vorum H, Hilpert J, Nykjaer A, Jacobsen C (1999). Megalin knockout mice as an animal model of low molecular weight proteinuria. Am. J. Pathol. 155(4): 1361-1370.

Longo DL, Fauci AS, Braunwald E, Isselbacher KJ (1998). Plasma cell disorders. Harrison's Principles of internal medicine 14th edition. New York: McGraw-Hill, p. 712.

Lund U, Rippe A, Venturoli D, Tenstad O, Grubb A, Rippe B (2003). Glomerular filtration rate dependence of sieving of albumin and some neutral proteins in rat kidneys. Am. J. Renal Physiol. 284(6): 226-234.

Maack T, Johnson V, Kau ST, Figueiredo J, Sigulem D (1979). Renal filtration, transport, and metabolism of lowmolecular- weight proteins: a review. Kidney Int. 16(3): 251-270.

Norden AG, Scheinman SJ, Deschodt-Lanckman MM, Lapsley M, Nortier JL, Thakker RV (2000). Tubular proteinuria defined by a study of Dent's (CLCN5 mutation) and other tubular diseases. Kidney Int. 57(1): 240-249.

Ohlson M, Sorensson J, Haraldsson B (2002). Glomerular size and charge selectivity in the rat as revealed by FITC ficoll and albumin. Am. J. Physiol. Renal Physiol. 279(1): F84-91.

Pappenheimer J, Renkin E, Borrero L (1951). Filtration, diffusion and molecular sieving through peripheral capillary membranes A contribution to the pore theory of capillary permeability Am. J. Physiol. 167: 13-46.

Parikh CR, Jani A, Melnikov VY, Fubel S, Edelsttein CL (2004). Urinary interleukin -18 is a marker of human acute tubular necrosis. Am. J. Kidney Dis. 43: 405-414.

Pratt DS, Kaplan MM (2003) Tests of the liver's biosynthetic capacity. In 11.3 edition: Up To Date.

Rostgaard J, Qvortrup K (2002). Sieve plugs in fenestrae of glomerular capillaries - site of the filtration barrier? Cells Tissues Organ. 170(2-3): 132-138.

Russo LM, Bakris GL, Comper WD (2002). Renal handling of albumin: a critical review of basic concepts and perspective. Am. J. Kidney Discov. 39(5): 899-919.

Stone RA (1989). Office evaluation of the patient with proteinuria; Postgraduate Med. 86(5): 241-244.

Tejler A Grubb AO, Schurek HJ, Neumann KH, Flohr H, Zeh M, Stolte H (1992). The physiological and pathophysiological basis of glomerular permeability for plasma proteins and erythrocytes. Eur. J. Clin. Biochem. 30(10): 627-637.

Tejler A, Grubb A (1992). Diagnostic value of analysis of cystatin C and protein HC in biological fluids: Clin.l Nephrol. 38 Suppl 1: S20-27.

Tencer J, Frick IM, Oquist BW, Alm P, Rippe B (1998). Size-selectivity of the glomerular barrier to high molecular weight proteins: upper size limitations of shunt pathways. Kidney Int. 53(3): 709-715.

Toto RD (1995). Conventional measurement of renal function utilizing, serum creatinine clearance inulin and para-aminohippuric acid clearance. Curr. Opin. Nephrol. Hypertension 4(6): 505-509.

Woo KT, Lau YK, Wong KS, Chiang GS (2000). ACEI/ATRA therapy decreases proteinuria by improving glomerular permselectivity in IgA nephritis. Kidney Int. 58(6): 248-591.

Zhongxin Z, Shahnaz S, William FK, Denise R, Tania ZD, Gilbert WG, Carl EM, Dick de Z, Barry MB, Steven MS (2005). Importance of Baseline Distribution of Proteinuria in Renal Outcomes Trials: Lessons from the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) Study. J. Am. Soc. Nephrol. 16: 1775-1780.