Proteinuria as a risk factor for the progression of chronic renal disease

Proteinurija – faktor rizika za progresiju hronične slabosti bubrega

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Introduction

The problem of proteinuria was recognised twenty-four hundred years ago, when Hippocrates noted the association between “bubbles on the surface of the urine” and kidney disease 1,2. Proteinuria is a common finding in adults in primary health care practice. A wide variety of conditions, ranging from benign to lethal, can cause proteinuria. Benign causes include fever, intense activity or exercise, dehydration, emotional stress, acute illness, and exposure to cold. More causes that are serious are kidney damages, like in glomerulonephritis and multiple myeloma 3.

A knowledgeable approach to this common condition and differentiation of benign and rarer, more serious, disorders causing proteinuria is required because the diagnosis has important ramifications for health, insurance eligibility and job qualifications 3.

Definition

Proteinuria is defined as urinary protein excretion greater than 150 mg/1.73 m²/24h, i.e. total urinary proteins over 11.3 mg/mmol creatinine (> 100 mg/g) 4−8.

Physiology and pathophysiology

Filtration

Plasma protein filtration is regulated by glomerular barrier, composed of endothelial cells, basement membrane, podocytes, (the epithelial cells with foot processes), and slit diaphragms located across the spaces between the epithelial foot processes. Under physiologic conditions, glomerular capillary wall is a size and charge selective barrier for passage of plasma proteins. Size-selective barrier consists of cylindrical pores and filtration gaps. According to their size, there are two classes of pores – small and large ones. Majority of pores are small, with diameters of 29–31 Å, responsible for low molecular weight plasma proteins (< 40 kD) filtration. The rest of pore population consists of a small number of large pores with diameter of 90/115 Å, which are sights of medium molecular weight plasma proteins (> 80 kD) filtration 9,10. Within intact glomerular capillary wall, besides two groups of pores, there are rare membrane shunts, large enough to allow passage of extremely large plasma proteins, as Immunoglobulin-M (molecular weight of 900 kD, diameter 120 Å) 9,10. Filtration gaps of 20−50 nm are the main mechanic barrier for passage of medium and high molecular weight plasma proteins 9,10.

Normally, due to presence of heparin sulfate and sialoglycoprotein, the basement membrane, endothelial cells and visceral epithelial cells are negatively charged, which represents charge selectivity barrier 9,10. To specify, negatively charged plasma albumin is repelled by the normal negative charge on the basement membrane and the intact endothelial cells. Circulating Immunoglobulin-G (IgG) has neutral or positive charge and is not repelled by negative charge on the basement membrane. Furthermore, immunoglobulin filtration is restricted by the size selective barrier of the membrane and the epithelial slit diaphragm. Loss of negative charge on luminal surface of glomerular capillary wall enables passage of plasma proteins 9,10.

Reabsorption

Under physiological conditions, more than 99% of filtered proteins are reabsorbed in proximal tubules by the process of endocytosis. Crucial role in reabsorption and degradation of filtered proteins play cells of proximal tubules S1-segment 11−13.
Proximal tubule epithelial cells exhibit two proven systems for protein reabsorption. Low capacity reabsorption system works under physiological conditions, and high capacity reabsorption system is activated when protein filtration is increased. Two glycoproteins, cubulin and megalin, are involved in reabsorption of filtered proteins. Cubulin (molecular weight of 460 kDa) is a glycoprotein in brush border of proximal tubule epithelial cells, which serves as a receptor for albumin, high density lipoproteins (HDL), apoprotein A-I and transferrin. Megalin (molecular weight of 600 kDa) is a transmembrane glycoprotein, receptor for low density lipoproteins (LDL), retinol-binding protein (RBP), β2-microglobulin, α1-microglobulin and vitamin D-binding protein (DBP). Immunoglobulin-G is reabsorped through IgG receptor (FcRn) located in brush border of proximal tubule epithelial cells. Under pathologic conditions, due to increased permeability of glomerular basement membrane and increased plasma protein filtration, reabsorption of proteins in tubules is increased, too. When maximum tubular capacity for protein reabsorption is exceeded, proteinuria occurs.

Classification of proteinurias according to protein species

Proteinuria is classified as prerenal, renal and postrenal. Prerenal proteinuria is consequence of enhanced production and increased concentration of low molecular weight plasma proteins, while integrity of glomerular and tubular anatomy and function is preserved. Proteinuria is prerenal if total protein excretion is > 300 mg/L, and sum of albumin, α1-microglobulin and IgG is < 30% of total excreted proteins. It can also be defined using ratio of albumin and total proteins (albumin/total proteins < 0.3). Renal proteinuria can be glomerular, tubulointerstitial and glomerulo-tubular.

Glomerular proteinuria arises due to injury of the glomerular basement membrane followed by loss of negative charge, increase in the number of larger non-selective pores, disruption and loss of the epithelial foot processes and increase of their permeability, with preserved anatomic and functional integrity of tubules. Glomerular proteinuria appears as selective or non-selective, differentiated with selectivity index (SI) – ratio of IgG clearance and albumin or transferrin clearance. Patients with IgG selective index ≥ 0.2 have “non-selective” proteinuria, while patients with the ratio < 0.2 have “selective” proteinuria. In glomerular disease with albuminuria > 500 mg/g of creatinine, IgG/albumin ratio is helpful in a differentiation of “selective” (IgG/albumin < 0.03) and “non-selective” proteinurias (IgG/albumin > 0.03).

Tubular proteinuria develops when anatomic and functional integrity of glomeruli is preserved, and anatomic and functional integrity of tubulointerstitium is disturbed. Glomerulo-tubular proteinuria, seen in progressive glomerular diseases, is result of injury of anatomic and functional integrity of both glomerules and tubules. In patients with nephrotic proteinuria (albumin excretion > 300 g/g creatinine; > 339 g/mol) separate contributions of tubulointerstitium and glomerules in genesis of proteinuria are estimated by calculation of corrected α1-microglobulin (“tubulointerstitial α1-microglobulin”).

$$\alpha_1\text{-microglobulin}_{\text{corrected}} = \frac{\alpha_1\text{-microglobulin}_{\text{measured}} - 4.7 \cdot e^{0.00022 \cdot \text{albumen}}}{1-microglobulincorrected}$$

Upper normal limit for albumin/creatinine ratio is 20 mg/g of creatinine, and for α1-microglobulin/creatinine 14 mg/g of creatinine. In patients with nephrotic proteinuria and corrected value of α1-microglobulin within reference range (≤ 14 mg/g of creatinine), tubular proteinuria is the result of tubular system overload by ultrafiltered proteins. In patients with primary glomerulopathy and nephrotic proteinuria corrected concentration of α1-microglobulin > 14 mg/g of creatinine indicates presence of tubulointerstitial injury.

Postrenal proteinuria is a consequence of postrenal bleeding. When albuminuria is > 100 mg/L, renal and postrenal causes of hematuria are differentiated by urine concentrations of α1-macroglobulin, immunoglobulin G and α1-microglobulin. Postrenal proteinuria exists when α1-macroglobulin/albumin ratio is > 0.02 and IgG/albumin ratio is > 0.2. Increased concentration of α1-microglobulin (α1-microglobulin/albumin ≥ 1.0) indicates interstitial cause of hematuria. Renal cause of hematuria is characterized by α1-macroglobulin/albumin ratio ≤ 0.02. In case of postrenal hematuria, ratios of α1-macroglobulin/albumin and IgG/albumin are > 0.02.

Influence of proteinuria on glomerular injury

Filtered proteins cause phenotypic alterations in glomerular mesangial and tubular epithelial cells that may have pathogenic significance. Due to increased permeability of glomerular capillary wall, lipoproteins accumulate in mesangium, LDL binds to specific receptors and stimulates mesangial cell proliferation and increases production and deposition of extracellular matrix proteins, leading to glomerulosclerosis. Also, LDL stimulates production of monocyte chemoattractant protein-1 (MCP-1), platelet-derived growth factor (PDGF) and endothelin-1 (ET-1) in mesangial cells. Released mediators act chemotactic on monocytes, increase production of extracellular matrix proteins (EMP) and lead to development of glomerulosclerosis. Cholesterol is synthesized within mesangial cells (mevalonate metabolic pathway) simultaneously with isoprenoids, i.e. metabolites of mevalonate (farnesyl pyrophosphate-FPP and geranylgeranyl pyrophosphate-GGPP) that activate transcription factors (by activation of protein-1 – AP-1, NF-κB, NF-interleukin-6 – NF-IL-6) and vasoactive and proinflammatory genes. This increases production and release of vasoactive and proinflammatory mediators that additionally accelerate development of glomerulosclerosis. Oxidized LDL – OxLDL stimulate renin release from juxtaglomerular cells, which increases production and secretion of angiotensin II. Angiotensin II stimulates mesangial cells proliferation, increased production and release of ET-1 and transforming growth factor β.
growth factor β1 (TGF-β1) that play key role in process of glomerular fibrous reaction. 

**Influence of proteinuria on tubulointerstitial injury**

Increased and prolonged protein reabsorption alters functional capacity of proximal tubules epithelial cells. Reabsorbed proteins are accumulated in lysosomes and degraded into amino acids. Due to lysozyme enlargement and cracking, proximal tubular and interstitial cells are exposed to lysozyme enzymes with cytotoxic and proinflammatory actions. Albumin and other proteins, accumulated within lumen of proximal tubules due to increased glomerular permeability, directly cause injury of proximal tubular epithelial cells (Figure 1). Cultured proximal tubule epithelial cells demonstrated that albumin stimulates mRNA transcription for pre-pro-endothelin-1, which turns into ET-1 under influence of endopeptidases and endothelin convertase. Approximately 80% of ET-1 is secreted via basolateral membrane of proximal tubular cells into renal interstitium. Endothelin-1 is chemoattractant for macrophages and stimulates fibroblast proliferation in interstitium and production of EMP, leading to inflammation and scarring of tubulointerstitium. Patients with chronic glomerular diseases and proteinuria over 2.0 g/24 h, increase production of ET-1, its excretion in urine, and exhibit increased ETb receptors within kidney. Albumin, but also IgG, HDL and transferrin stimulate mRNA transcription for pre-pro-endothelin-1, along with increased production and release of ET1. Albumin also stimulates mRNA transcription for monocyte chemoattractant protein-1 (MCP-1), RANTES (Regulated upon Activation Normal T-cellExpressed and Secreted) and osteopontin, cytokines released through basolateral membrane of proximal tubular epithelial cells into renal interstitium and acting chemotactically upon monocytes and T-lymphocytes. Activated infiltrative cells (mono-
mesenchymal indices such as FSP-1, VIM and α-cadherin. Angiotensin II stimulates proximal tubules epithelial cells to increase production and release of TGF-β, contributing additionally to scarring of renal parenchyma. Angiotensin II is filtered through glomerular capillary wall, and on surface of proximal tubular epithelial cells under action of aminopeptidase N it is decomposed to angiotensin IV. Angiotensin IV, via AT1 receptors in proximal tubules epithelial cells, increases production and secretion of PAI-1 (plasminogen-1 activator inhibitor), which blocks degradation of EMP in renal interstitium and accelerates process of tubulointerstitial scaring.

Tubulointerstitial scaring process develops through three stages. In stage 1, cytokines, chemokines and growth factors from kidney cells and infiltrated mononuclear cells are released, and number of fibroblasts in interstitium is increased. Two mechanisms contribute to increase of fibroblasts number, a central event in tubulointerstitial scaring: proliferation of fibroblasts constantly present within interstitium and transformation of tubular epithelial cells into fibroblasts.

Under pathologic conditions, in process of tubulointerstitial scaring, fibroblasts are activated, they express de novo α-smooth muscle actin (α-SMA), interleukin-1 (IL-1) and basic fibroblast growth factor-2 (bFGF-2), their proliferative capacity increases together with capacity to produce extracellular matrix protein. Several clinical and experimental studies have proven that TGF-β1 and bFGF-2 are major cytokines in the process of renal parenchyma scaring.

Transdifferentiation (change) of tubular epithelial cells into mesenchymal cells (fibroblasts) and their transfer to kidney interstitium also contribute to increased number of fibroblasts within kidney interstitium and scaring of kidney parenchyma. De novo expression of fibroblast specific protein-1 (FSP-1) was proven in tubular epithelial cells in initial stage of renal parenchyma scaring. This indicates possibility for transformation of epithelium into mesenchyme, or epithelial-mesenchymal transdifferentiation. Transdifferentiation is defined as ability of proximal tubular epithelial cells to gain mesenchymal phenotype, characterized by downregulation of epithelial index cytokeratine and upregulation of mesenchymal index vimentin. Epithelial-mesenchymal transdifferentiation (EMT) includes structural changes in epithelial cells; they assume spindle shape, expressions of epithelial indices such as cytokeratines, E-cadherin, ZO-1 and syndecan-1 are reduced, expression of mesenchymal indices such as FSP-1, VIM and α-SMA is increased, and changes in collagen production occur – predominance of collagen type I/III.

Epithelial-mesenchymal transdifferentiation can be induced by cytokines such as TGF-β1 and bFGF-2. Basic fibroblast growth factor-2 together with TGF-β1 causes reduced expression of cytokeratine and E-cadherin in epithelial cells of proximal tubule, while production of FSP-1 and α-SMA increases 6–8 times. In EMT, tubular epithelial cells lose apical-basal polarity, begin to express mesenchymal indices, such as α-SMA and FSP-1, and get ability to migrate. Extracellular matrix proteins also influence the EMT process. It is established that interruption of collagen type IV, the main component of tubular basement membrane, provokes EMT. Interruption of tubular basement membrane (BMT) is an event that could transform tubular epithelial cells into mesenchymal cells that produce extracellular matrix proteins lifelong.

The second stage of tubulointerstitial scaring is characterized by fibroblast activation. During scaring process, interstitial fibroblasts change their phenotype, express new molecules like α-smooth muscle actin, which is under physiologic conditions expressed in smooth muscle cells of renal blood vessels. From that point, those cells are termed as “myofibroblasts”, able to increase production of EMP, such as fibronectin, collagen types I, III, IV and V Number of α-SMA positive cells in interstitium correlates to the degree of scaring and kidney function. Activated myofibroblasts proliferate and increasingly produce EMP responsible for widening of tubulointerstitial space and consequent loss of kidney function.

However, creation of inflammatory matrix is not characterized only by production of EMP, but also by reduced matrix degradation. Matrix metalproteinases (MM), such as interstitial collagenases, gelatinoses and stromelysin/transin, are responsible for degradation of extracellular matrix. Their activity is regulated by tissue inhibitors of matrix metalloproteinase (TIMP), which block all active forms of MM by direct binding. There are three different metalloproteinase tissue inhibitors that degrade EMP. It is established that proteinuria leads to increased production and secretion of TIMP-1. Another important enzyme in control of extracellular matrix protein degradation is the PAI-1, which inhibits the proteolytic conversion of plasminogen into plasmin. Plasmin has ability to directly degrade variety of extracellular matrix proteins and to activate latent metalloproteinases that degrade matrix. This stage is followed by increased production and reduced degradation of matrix proteins, which additionally increases deposition of EMP (“generative stage of extracellular matrix proteins”).

The third stage, the stage of postinflammatory production of extracellular matrix protein, is characterized by production of EMP despite cessation of inflammatory stimulus. Possible mechanisms responsible for these processes include ability of survived epithelial cells to produce and secrete cytokines contributing to scaring process, such as TGF-β1 and PDGF; ability of tubular epithelial cell for transformation into mesenchymal cells that increasingly produce extracellular matrix proteins; presence of interstitial mononuclear cells that persist within interstitium and continuously produce and secrete cytokines that contribute to scaring and stimulate production of interstitial matrix.

Influence of proteinuria on lipid metabolism disturbance

In proteinuric patients, lipid metabolism disturbance is a consequence of increased lipid and apoprotein production

in liver and reduced clearance of chylomicrons, very low density lipoproteins (VLDL), intermediary lipoproteins (IDL) and LDL. Hypoalbuminemia, developed due to increased urinary protein loss and albumin degradation in proximal tubules epithelial cells, is responsible for reduction of plasma oncotic pressure, which promotes fluid shift in interstitial space and stimulates liver production of lipoproteins and apoproteins.

Also proven in patients with nephrotic syndrome is reduced activity of lipoprotein lipase (LPL) and lecithin-cholesterol acyltransferase (LCAT). Reduced LPL activity is a consequence of increased urinary loss of cofactor apo C-II and HDL particles, which transport apo C-II to VLDL lipoproteins, and increased loss of gluconsaminoglycans (heparan sulphate proteoglycan) that bind enzyme to endothelium. In patients with nephrotic syndrome, apo C-II is lost via urine as a free protein or coupled with HDL. Additionally, in these patients plasma apoprotein C-III concentration is increased, blocking LPL activity. Ratio of apo C-II/apo C-III is significantly reduced in these patients. Besides reduced LPL activity, it is established that reduced plasma oncotic pressure affects binding of LPL to capillary endothelium, disturbing VLDL lipoprotein clearance.

In nephrotic syndrome, there is a perturbation of HDL (HDL₃) maturation under influence of LCAT. Under physiologic conditions, lysolecithin, released during cholesterol esteriﬁcation, binds to albumin in circulation. When proteinuria and hypoalbuminemia occur, concentration of free lysolecithin rises and blocks LCAT activities, suppresses cholesterol esteriﬁcation within HDL particles and therefore blocks turnover of HDL₃ into HDL₂. Disturbance of HDL maturation reduces trafﬁc of apo C-II to VLDL and blocks degradation of triglyceride-rich lipoproteins. Furthermore, mature HDL also transport numerous apoproteins and several cofactors. One of these apoproteins is apo C-II, endogenous LPL-activity activator.

Nephrotic syndrome patients show increased serum concentrations of total cholesterol, LDL and Lp (a) and apolipoprotein B and apolipoprotein E, as well as levels of triglycerides and VLDL, especially if serum albumin level is below 20 g/L (10–20 g/L). Depending on proteinuria severity HDL are normal, increased or reduced.

**Therapeutic aspect of proteinuria**

Chronic renal diseases exhibit tendency of progression to end-stage chronic renal failure. Processes responsible for progression of chronic renal disease take place in glomeruli and tubulointerstitium. Primary renal disease lead to critical loss of nephrons, followed with functional adaptation in the remaining nephrons characterized by increased hydrostatic pressure in glomerular capillaries (glomerular hypertension), leading to increase in filtration capacity of individual nephrons (SNGFR). Increased glomerular capillary hydrostatic pressure increases plasma protein filtration, and enhanced and prolonged reabsorption of plasma proteins activates vasoactive and proinflammatory genes in proximal tubule epithelial cells.

Proteinuria is an independent risk factor for development and progression of chronic renal disease, and an independent predictor for a degree of glomerular filtration deterioration. In patients with nephrotic proteinuria (proteinuria > 3.5 g/24h) three-year follow up established the degree of glomerular filtration deterioration ≥ 10 ml/min/1.73 m²/year.

Hydrostatic pressure lowering in glomerular capillary using angiotensin I convertase blockers (ACE inhibitors), angiotensin II receptor (ARA) blockers and low dietary protein intake (0.8 g/kg of body weight per day), can reduce proteinuria, prevent progressive scarring of renal parenchyma and deterioration of renal function. Their performances are based on reduction and prevention of angiotensin II production.

Renin-angiotensin system blockade using ACE inhibitors and/or ARA blockers is essential in reduction of proteinuria and slowing chronic renal failure progression, and is indicated in patients with incipient or clinically manifest diabetic nephropathy and in non-diabetic patients with proteinuria > 0.5–1.0 g/24h, regardless of blood pressure and before occurrence of renal insufﬁciency (serum creatinine < 1.5 mg/dl or < 133 μmol/L).

Statins have a signiﬁcant role in prevention of chronic renal disease progression. In patients with chronic renal disease and proteinuria, HMG-CoA reductase blockers reduce concentrations of total and LDL cholesterol, increase HDL cholesterol concentrations, reduce urinary protein loss and decelerate chronic renal disease progression. Statins achieve these actions through blocking the production of cholesterol and isoprenoides (mevalonate metabolites), included in early activation of transcription factors (NFkB), which lead to gene expression in mesangial and epithelial cells of proximal tubules, necessary for the process of inﬂammation and scaring in renal parenchyma. In patients with chronic renal disease and proteinuria, target level of LDL cholesterol should be < 2.6 mmol/L (< 100 mg/dl).

Application of non-selective ET-1 receptor antagonists (ETA/ETB) also prevents remodeling of renal parenchyma and exhibits protective action on renal function. Combination of angiotensin II and ET-1 receptor blockers enhances protective action against reduction of glomerular ﬁltration intensity and slows down development of end-stage chronic renal disease. Blockers of angiotensin I convertase and/or angiotensin II receptor antagonists reduce plasma protein trafﬁc through glomerular capillary wall, and statins and ET-1 receptor antagonists prevent consequences of protein trafﬁc and their reabsorption on remodeling process of glomerules and tubulointerstitium.

**Conclusion**

Proteinuria leads not only to glomerular injury and development of glomerulosclerosis, but also to injury of tubulointerstitium.

Timely diagnosis of proteinuria, identifying proteinuria causes and application of appropriate therapy enable to slow down progression of chronic renal disease and to prevent development of end-stage chronic renal disease.
REFERENCES


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