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Review

Low Molecular Weight Proteinuria - An Update

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Abstract

Low molecular weight proteins (LMWP) are those proteins with molecular weight below 67,000 Da, which can freely pass through the glomerular sieve. This passage is dependent on the size, configuration and charge of the protein molecule. In tubular disorders (e.g. Fanconi syndrome) there is defect in the tubular handling of LMWP and they appear in the urine in measurable concentrations. Determination of the LMWP in the urine is the basic diagnostic tool for tubular diseases. Urinary protein electrophoresis was the basic method for determination of LMWP but with the discovery of beta-2 microglobulin (molecular weight 11,600 Da) ensued a new era in urinary protein chemistry. There is a growing list of diseases with LMWP as a principal feature. Many of these diseases are genetic in origin. With huge advance in molecular genetic techniques (e.g. next generation sequencing) molecular basis of these diseases has already been elucidated as well as pathophysiological mechanisms which lead to occurrence of LMWP. Besides genetic diseases affecting the kidney tubules, there are also acquired diseases which can affect proximal tubules resulting in LMWP and additional tubular defects as in the case of drug induced Fanconi syndrome. LMWP is often unrecognized in the busy clinical practice. In this review we will focus on the pathophysiological events leading to LMWP, assays for its detection and various clinical disorders presenting with LMWP.

Keywords: proteinuria, molecular weight, proximal tubule, Fanconi syndrome, genetics

Introduction

Proteinuria was known as an associated feature of kidney disease many centuries ago, but the works of Butler and Flynn led to the discovery that tubular disorders were associated to particular pattern of proteinuria [1,2]. Electrophoresis of the urinary proteins enabled separation of the proteins according to the molecular weight (Figure 1). This pattern associated with tubular disorders was termed tubular or low molecular weight proteinuria (LMWP). There is a growing list of diseases with LMWP as a principal feature. Many of these diseases are genetic in origin. With huge advance in molecular genetic techniques (e.g. next generation sequencing) molecular basis of these diseases has already been elucidated as well as pathophysiological mechanisms which lead to occurrence of LMWP. Besides genetic diseases affecting kidney tubules, there are also acquired diseases which can affect proximal tubules resulting in LMWP and additional tubular defects as in the case of drug induced Fanconi syndrome. LMWP is often unrecognized in the busy clinical practice. The first step in evaluation of a patient with proteinuria is its quantifi-

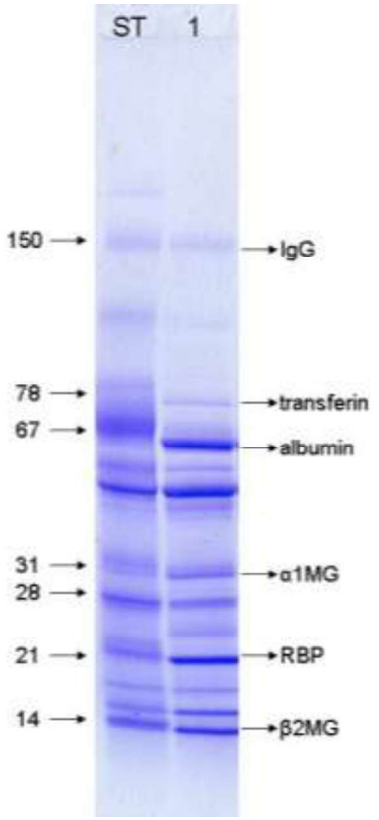

Fig. 1. SDS-PAG electrophoregram of a patient with mixed glomerulotubular proteinuria (lane 1). ST-standard

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cation and typization after exclusion functional causes (postural, exercise induced, febrile proteinuria) [3-9].
In this review we will focus on the pathophysiological events leading to LMPW, assays for its detection and various clinical disorders presenting with LMWP.

Physiologic basis

LMW proteins are those proteins with molecular weight below 67,000 Da, which can freely pass through the glomerular sieve. This passage is dependent on the size, configuration and charge of the protein molecule [10-12]. After filtration in the glomeruli LMW proteins are reabsorbed completely in the proximal tubule (99.9%) through the endocytosis and then catabolyzed to aminoacids in the lysosomes. In tubular disorders (e.g. Fanconi syndrome) there is a defect in the tubular handling of LMWP and they appear in the urine in measurable concentrations. Determination of the LMWP in the urine is the basic diagnostic tool for tubular diseases. As already mentioned urinary electrophoresis was the basic method for determination of LMWP [3]. With the discovery of beta-2 microglobulin (molecular weight 11,600 Da) ensued a new era in urinary protein chemistry [13]. The discovery of other urinary protein markers has led to improvement of classification (typization) of proteinuria. Retinol binding protein (RBP) was discovered in 1968. It is a carrier of vitamin A, molecular weight of 21,200 and is synthesized in the liver [13]. RBP is bound to the prealbumin and only the small free fraction (5%) is filtered through the glomeruli. The third LMWP alpha 1 microglobulin (A1M) was discovered in 1975 with molecular weight which varies from 24,800 to 31,000 Da [13]. Other LMW are ribonuclease, free kappa light chains of immunoglobulins and urine protein 1 (UP1), N-acetyl-[3-D-glucosaminidase], brush border enzymes (e.g. alanine aminopeptidase), the Tamm-Horsfall glycoprotein etc.

Assay methods

Urinary electrophoresis was the basic method for detection of LMWP. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) improved the detection of LMWP, but it lacked sufficient sensitivity. Modification of the techniques (silver staining) and western blotting resulted in better protein identification and quantification [14]. Nowadays there are many assays for the measurement of LMW proteins in urine. This measurement is based on enzyme-linked immunosorbent assay (ELISA) or turbidometry (nephelometry). Commercial kits are available for LMW proteins. Reference ranges for urine concentrations of LMW proteins in both adults and children have been published and should be expressed as ratio with urinary creatinine. One should have in mind that referent values for infants up to 6 months of age are much high due to the immaturity of the tubular function. Particular attention should be devoted to accurate collection and proceeding of samples for the analysis. B2M is unstable in the acidic urine which enhances its degradation at a pH less than 5.5 [13]. This degradation is both time and temperature dependent, especially during the overnight collections. Therefore, a second morning urinary sample is most suitable for analysis. Immediate alkalization of the urine after collection is also important to prevent degradation. RBP and AIM are stable in urine of physiological pH at room temperature, but RBP is unstable below pH 5.0 when stored frozen at -20^{o}C, in contrast to A1M.

Competition for reabsorption

Proteinuria can be divided into 3 basic patterns: glomerular, tubular or mixed types by quantitative assay of albumin and B2M in urine. However, this relationship is not applicable in disorders with heavy proteinuria as in the case of nephrotic syndrome. There is clear evidence from animal studies for competition between albumin and LMWP for reabsorption in the proximal tubule [15]. There is inverse relation between the LMW protein excretion in urine and GFR, occurring in both glomerular and tubular diseases. This may not be applied for advanced chronic kidney disease (GFR <30 ml/min per 1.73 m2) where the tubular reabsorptive capacities are insufficient for the elevated plasma levels of LMWP.

Clinical Disorders

Low molecular weight proteinuria is the hallmark of tubular and tubulointerstitial disorders, but it can also be seen in glomerular disease, kidney transplantation and diabetes mellitus.

Disease with isolated low molecular proteinuria

Imerslund-Grasbeck syndrome (IGS) is a rare genetic autosomal recessive disorder characterized by the triad: low molecular weight proteinuria, megaloblastic anemia and vitamin B_{12} deficiency [16]. The disease occurs after the fourth month of age and additional clinical features are failure to thrive, recurrent respiratory and intestinal infections, mild neurological signs and symptoms. Proteinuria is persistent and does not respond to treatment with vitamin B12. The renal prognosis is excellent. The disease is a result of mutation in two genes-cubilin (*CUBN)* and amnionless (*AMN*). Both proteins are expressed in the small intestine as well in the renal proximal tubular cells. In the kidneys they interact with the multi-specific endocytic receptor megalin allowing the reabsorption of a panel of filtered plasma proteins such as albumin, vitamin D-binding protein, apolipoprotein A-I, and transferrin.

Donnai-Barrow syndrome or facio-oculo-acoustico-renal syndrome (MIM 222448)) is characterized by typical craniofacial anomalies (hypertelorism, bulging eyes), corpus callosum agenesis, developmental delay, high grade myopia, sensorineural deafness and low molecular weight proteinuria [17]. This is a very rare autosomal recessive disorder due to mutations in the low density lipoprotein receptor-related protein 2 gene LRP2. LRP2 encodes megalin, a multi-ligand endocytic receptor important for receptor-mediated endocytosis (RME). Besides the kidney, megalin is expressed in many absorptive epithelia, including the neuro-epithelium. During the brain development megalin mediates neural tubule specification by acting as a clearance receptor of various ligands, such as bone morphogenic protein 4 from extra-embryonic fluids [18]. During optic nerve development, megalin modulates sonic hedgehog abundance and enables the recruitment of oligodendrocyte precursors [19]. Megalin is also expressed in the retinal pigment epithelium and nonpigmented ciliary body epithelium [20]. These observations explain the important role of the megalin in brain and eye development and also explain the disease phenotype.

Renal Fanconi syndrome is a heterogeneous entity due to different causes. It can be isolated or associated with affection of multiple organs and systems as in the case of cystinosis and mitochondrial cytopathies. It can be congenital (genetic) or acquired, transient or persistent, with preserved GFR or with progression to end-stage renal failure [21]. Some patients may have mild affection of the proximal tubular functions (Dent disease) or severe dysfunction (cystinosis). Genetic causes of Fanconi syndrome are given in Table 1.

Table 1. Genetic causes of Fanconi syndrome

Disease	Gene	Additional clinical features
Cystinosis	*CTNS*	Multiple organs affected, corneal cystine crystals
Oculocerebrorenal syndrome of Lowe	*OCRL*	Congenital cataracts, neurological deficit, kidney failure
Dent-1	*CLNC5*	Hypercalciuria, nephrocalcinosis, stones, kidney failure
Dent-2	*OCRL*	Hypercalciuria, nephrocalcinosis, stones, kidney failure, peripheral cataracts, mild intellectual disability
Tyrosinemia	*FAH*	Hepatic dysfunction, liver cancer, growth retardation
Wilson disease	*ATP7B*	Liver dysfunction, neurological abnormalities
Galactosemia	*GALT*	Jaundice, liver dysfunction, encephalopathy
Congenital Fructose Intolerance	*ALDOB*	Hypoglycemia, vomiting, hepatomegaly
Fanconi–Bickel syndrome	*GLUT2*	Hepatosplenomegaly, hypo-, hyperglycemia, poor growth, rickets
ARC syndrome	*VPS33B, VIPAR*	Arthrogryposis, cholestasis
Mitochondrial cytopathies	Multiple mitochondrial and nuclear DNA mutations	Multiorgan dysfunction
MODY1	*HNF4A*	Neonatal hyperinsulinism, Maturity-onset of diabetes in the young
FRTS1	Not known, linked to chromosome 15	Kidney failure
FRTS2	*SLC34A1*	Bone fractures due to hypophosphatemia
FRTS3	*EHHADH*	Preserved GFR

ARC syndrome- Arthrogryposis-renal dysfunction-cholestasis syndrome' FRTS-Fanconi Renotubular Syndrome

Aquired Fanconi syndrome in majority of cases is due to drug toxicity [22]. The modern medicine is characterized by the expansion of the pharmaceutical industry and creation of new modern drugs for treatment of diseases which were considered incurable such as cancer, epilepsy or HIV infection. However, many of these drugs have potential to damage the proximal tubules [23-25]. These drugs are extracted from the blood stream into the proximal tubular cells through a number of organic transporters expressed on the cell surface [26,27]. This leads to high intracellular concentration of these drugs, which explains their toxic effect. The precise prevalence of the drug-induced Fanconi syndrome is not known, because these drug-adverse effects may be mild and not always recognized and reported.

Platinum-containing compounds (cisplatin and carboplatin) are widely used anticancer drugs for treatment of adults as well children. Ifosfamide has similar chemical structure to cyclophosphamide, which is not nephrotoxic. The toxicity of ifosfamide is explained by its rapid uptake into tubular cells through the action of cationic organic transporters and its metabolism to toxic chloracetaldehyde [28]. In majority of cases toxicity from cisplatin and ifosfamide is reversible, but in some individuals can persist for years and lead to chronic tubulopathy.

Anti-viral drugs, nucleoside reverse transcriptase inhibitors (NRTIs) and nucleotide reverse transcriptase inhibitors (NtRTIs) designed a new revolutionary era in treatment of children and adults with HIV infection. Both groups of drugs expose tubular nephrotoxicity due to their high intracellular uptake by the organic transporters [29-34]. Tenofovor is a newer agent for treatment of HIV but also hepatitis B infection. Although initial safety studies did not show adverse effect on the glomerular filtration rate, further clinical reports described its tubulotoxic effect leading to Fanconi syndrome. It is estimated that serious toxicity ensues in less than 1% of patients [34].

Although aminoglycoside antibiotics (gentamycin, tobramycin and amikacin) are well known causes of drug-induced Fanconi syndrome, it seems that this adverse effect is nowadays rarely seen because of the increased awareness of the medical professionals and better monitoring during the administration of these drugs [35-39]. Tetracycline-induced Fanconi syndrome was described in classic textbooks, and has not been seen any more particularly in children because of the highly restrictive use of this drug [40-42].

Valproic acid is a widely used drug for treatment of epilepsy and mood disorders, particularly in children. Fanconi syndrome is described with the use of valproic acid, particularly in children with severe motor and intellectual disabilities [43-45]. Animal studies have revealed that pathomechanism of the tubular injury is related to the oxidative stress and mitochondrial dysfunction induced by valproic acid [46].

Other diseases

Many infectious agents can cause tubulointerstitial diseases resulting in tubular proteinuria and secondary Fanconi syndrome. Also vasculitides and autoimmune disorders can affect the proximnal tubules resulting in LMWP and other defects [47, 48, 49]. Here we should mention TINU syndrome (tubulointerstitial nephritis associated with uveitis) [50, 51]. Many papers reported about the presence of low molecular weight proteinuria in children with vesicoureteral reflux, idiopathic nephrotic syndrome, diabetic nephropathy and after kidney transplantation, but there is no clear evidence that LMWP is a predictor of the outcome and effect of the therapy in these diseases [52-59]. It seems that LMWP reflects the presence of the tubulointerstitial histology changes which can influence the disease course.

Transitory LMWP proteinuria may be seen in children with distal renal tubular acidosis [60-62]. The plausible explanation for this phenomenon is long-lasting hypokalemia and acidosis which can impair proximal tubular transporters resulting in LMWP and other defects. With metabolic compensation LMWP ceases in these patients.

Conclusion

Low molecular weight proteinuria may be found in many genetic and acquired kidney diseases. Often it is unrecognized in the busy clinical practice and may lead to unnecessary treatment with cytostatic agents and ACE inhibitors. Therefore, appropriate typization and classification should be an initial step in evaluation patients with proteinuria.

Conflict of interest statement. None declared.

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