

## CHAPTER

# 20

## *Proteinuria- Long Term Complications*

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### **Introduction**

Patients with diabetes or hypertension with microalbuminuria (MA)/ proteinuria are prone to CV risk and progressive renal damage. This risk increases progressively with increasing levels of proteinuria.<sup>1-3</sup> MA generally increases the relative risk of CV mortality by 1.3 and macroproteinuria by 2.4. 10-20% of hypertensive, 30-40% of diabetics and 5-7% of healthy population has MA. The CV risk exists even in seemingly healthy population with MA (without diabetes or hypertension). A population based British study on ischemic stroke reported a hazard ratio of 1.48 in patients with MA and 2.46 for macroproteinuric patients. Data from population based studies from Korea, Japan and from UK shows that 10-12 % of population has MA and approx 1% have proteinuria.<sup>4-6</sup> Reduction in proteinuria in all subgroups is associated with reduction in this risk. Hence MA or proteinuria provides an excellent early target for intervention.

### **Pathophysiology of proteinuria**

Glomerulus acts like a filtration barrier to medium and high mol wt proteins and only allows low mol wt proteins (mol wt < 20,000) to get freely filtered. Physiologically, the proteins excreted include mucoproteins like Tamm-Horsfall proteins, blood group proteins, albumin, mucopolysaccharides,

immunoglobulins, and very small amounts of hormones and enzymes. The low molecular weight proteins and little amount of albumin (mol wt 68,000) that are filtered from plasma into early tubular fluid are almost completely reabsorbed and catabolised in the proximal tubules. Thus the daily urinary protein excretion is less than 150 mg. Of the 150 mg proteins excreted less than 30 mg is albumin, around 20% are low mol wt proteins and rest 50% is Tamm Horsfall protein. Proteinuria is classified as transient, orthostatic and persistent. Transient proteinuria develops following exertion or in febrile settings and doesn't have any serious connotation. Orthostatic proteinuria is seen in young adults and is posture related. Two urinary protein collections i.e. comparison of 16 hours day time and 8 hours night time collection gives the diagnosis, since night collection shows absence of protein excretion. Prolonged follow up of these patients (over 20 years) shows that outcome of these patients is benign.<sup>7</sup> In next paragraphs we shall be discussing only about persistent proteinuria.

The term microalbuminuria (MA) implies albumin excretion of 20-200 ug/minute i.e. 30-300 mg/day. Protein excretion over this limit is termed as proteinuria. It has been observed that even in normoalbuminuric patients; those with higher normal have higher risk than those with lower normal albuminuria. It has been therefore

suggested by Ruggenti and Remuzzi<sup>8</sup> that the term microalbuminuria should be abandoned in favour of albuminuria since in true terms there is no safe lower limit/ cut off limit to MA. With adequate control of blood pressure or blood sugar over half of the patients either continue to have MA or become normoalbuminuric. One should be labelled to have MA if at least 2 of 3 readings over 3-6 m period show presence of albumin. Conventionally proteinuria is tested by 24 hour urine collection but now it is recommended to have spot protein/creatinine ratio as it is equally or even more reliable than 24 hour collection results.<sup>2,8,9</sup>

Pathologically, proteinuria can be divided into 3 categories: glomerular proteinuria, tubular proteinuria and overload proteinuria. Glomerular proteinuria results from an increase in the permeability of glomerular capillary wall to macromolecules caused due to glomerular disease and is usually over 2 gm/day. Tubular proteinuria results from reduced reabsorption of proteins that are normally present in the glomerular filtrate or from excretion of proteins derived from injured tubular epithelial cells and is usually less than 2 gm. It is generally caused due to diseases of tubulo-interstitium. Overload proteinuria is due to an excess of low molecular weight proteins, which are normally reabsorbed by the proximal tubules, but are sometimes overwhelmed due to excessive production. These proteins are most often immunoglobulin light chains (in plasma cell dyscrasias), lysozyme (in Myelomonocytic leukemia), myoglobin (rhabdomyolysis) or hemoglobin (intravascular hemolysis).

Quantitatively proteinuria can be divided into (i) Microalbuminuria (ii) Nephrotic and (iii) sub nephrotic proteinuria. Malb actually is a marker of endotheliopathy i.e. generalised endothelial damage. Proteinuria on the other hand implies established renal damage, which is a manifestation of variety of renal limited or systemic diseases. If protein excretion exceeds 3.5 gms/day it is termed nephrotic proteinuria. This is associated with hypoalbuminuria, hyperlipidemia and edema. A

number of proteins are also lost and it leads to a variety of metabolic disturbances due to deficiency of thyroxine binding globulin, cholecalciferol binding protein, transferrin, and metal binding proteins. It also produces a hypercoagulable state due to urinary losses of antithrombin III, reduced levels of proteins S and C, hyperfibrinogenemia and enhanced platelet aggregation. IgG deficiency may lead to defects in immunity.<sup>1,2,7,9</sup>

### **Link between proteinuria and CVD risk**

Several studies show that CV risk is higher in patients with malb or proteinuria. The nature of the link between microalbuminuria and cardiovascular risk remains poorly understood. Hypothesis can be (i) Does malb cause atherothrombotic disease and therefore predisposes to CV risk or (ii) atherothrombotic disease causes malb which is just a marker of generalised atherosclerosis or (iii) both malb and atherothrombosis which cause endothelial dysfunction have some common unidentified denominator which is responsible for CV risk.

There is no strong evidence that microalbuminuria causes atherothrombosis or that atherothrombosis causes microalbuminuria. Many studies have tested the hypothesis for a common link between microalbuminuria and cardiovascular disease but, again, have found no strong evidence. MA in type 1 and type 2 diabetes is usually accompanied by endothelial dysfunction with regard to the regulation of hemostasis, fibrinolysis, leukocyte adhesion, and NO synthesis and/or availability; as estimated by plasma levels of endothelial function markers such as von Willebrand factor, tissue-type plasminogen activator, soluble vascular cell adhesion molecule-1, and soluble E-selectin and by endothelium-dependent vasodilation in response to increases in flow or to agonists such as cholinergic agents. It is believed that endothelial dysfunction probably is common denominator. Endothelial derived vasodilator NO production is impaired in MA patients with cardiovascular disease regardless of whether diabetes is present. Indeed, several studies have shown that endothelial dysfunction

precedes and predicts the onset of MA in individuals without and with diabetes.<sup>10-13</sup> This in turn raises the question of how endothelial dysfunction could cause MA. Albumin is a relatively large, negatively charged protein (molecular weight 69 kD; size 36 Å). The filter through which albumin must pass before entering the urine, the glomerular capillary wall, is size and charge selective. MA is thought to be a consequence of an increased albumin leakage through the glomerular capillary wall as a result of increased permeability of the wall, an increased intraglomerular pressure, or both. For example, hyperglycemia and high BP are generally accepted risk factors for development of microalbuminuria. Both can increase intraglomerular pressure. In addition, hyperglycemia can alter the charge selectivity of the glomerular capillary wall, thereby increasing its permeability. In a healthy kidney, > 99% of filtered albumin is reabsorbed in the proximal tubules. Some data suggest that MA, at least in patients with type 2 diabetes, is associated not only with increased glomerular protein passage but also with an absence of a compensatory increase in tubular reabsorption of albumin. A pronounced increase in albumin filtered by the glomerulus will lead to excessive supply of albumin to the renal tubule, eventually exceeding tubular reabsorptive capacity, and thus to increased albumin excretion in the urine.<sup>11</sup>

Theoretically, endothelial dysfunction could cause albuminuria by increasing glomerular pressure and glomerular barrier permeability. Previously, glomerular barrier permeability was thought to depend mainly on glomerular basement membrane composition and slit diaphragm structure. Recent evidence, however, has pointed toward a more important, direct role of the endothelium in determining permeability to albumin.<sup>12,13</sup> In particular, the glycocalyx that fills the endothelial fenestrae seems to be important for glomerular size and charge selectivity. Abnormalities in the endothelial glycocalyx may contribute to (micro)albuminuria but also have been implicated in the pathogenesis of atherosclerosis, thus providing a potential direct link between albuminuria and cardiovascular disease. In particular, this recent knowledge of a possible common endothelial mechanism for increased glomerular

albumin leakage and generalized vascular disease sheds new light on the concept that microalbuminuria reflects a systemic transvascular leakage of albumin, which might predispose to greater penetration of atherogenic lipoprotein particles into the arterial wall—the Steno Hypothesis. Atherothrombosis currently is understood as a process in which endothelial dysfunction and chronic, low-grade inflammation are important early events. Indeed, chronic, low-grade inflammation can be both cause and consequence of endothelial dysfunction, and the two are tightly linked. Chronic, low-grade inflammation can be assessed by measurement of plasma levels of C-reactive protein and cytokines such as IL-6 and TNF- $\alpha$ . Studies using such markers have shown that, regardless of the presence of diabetes, chronic, low-grade inflammation is associated with the occurrence and the progression of MA and with risk for atherothrombotic disease. At present, the most likely possibility is that a common pathophysiologic process, such as endothelial dysfunction, chronic low-grade inflammation, or increased transvascular leakage of macromolecules, underlies the association between microalbuminuria and cardiovascular disease, though more and prospective studies of these hypotheses are needed.<sup>10-13</sup>

Such associations between urinary albumin excretion and cardiovascular disease extend beyond microalbuminuria. For example, in a 10-yr follow-up study showed that macroalbuminuria (*i.e.*, albumin excretion above the microalbuminuria threshold) was associated with an approximately three-fold increased cardiovascular risk among hypertensive men. In the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) study, any decrease in albuminuria was strongly related to decreased risk for cardiovascular as well as renal outcomes.<sup>14-16</sup>

### **Mechanism of progressive renal damage**

Several lines of evidence suggest a role of plasma proteins or protein-associated factors in chronic tubulointerstitial damage. Besides the glomerular sclerosis caused due to proteins interstitium bears

the brunt. *In vitro* data shows that in monolayers of proximal tubular cells, the plasma proteins (albumin, IgG & transferrin) induce synthesis of vasoconstrictor peptide endothelin-I (ET-I), a mediator of progressive renal injury by virtue of its ability to stimulate renal cell proliferation and extra-cellular matrix production and to attract monocytes.<sup>17</sup> Investigations of the molecular mechanisms underlying chemokine upregulation in proximal tubular cells on protein challenge was found to be due to activation of transcriptional NF- $\kappa$ B and reactive oxygen as second messenger since proteinuria elicited rapid generation of hydrogen peroxide in human proximal tubular cells and this effect is prevented by antioxidants. Despite evidence that albumin overload elicits several responses by tubular cells *in vitro*, the albumin itself may not be toxic.<sup>18,19</sup> Compounds that are bound to albumin such as free fatty acids (FFA) have been implicated as causing pro-inflammatory activation and injury of proximal tubular cells. It has been found that tubular metabolism of albumin bound fatty acids could generate macrophage chemotactic activity; where as lipidated albumin produces little such activity. Local recruitment of macrophages by tubular cells that are loaded with ultrafiltered plasma proteins may contribute to interstitial fibrosis by engaging matrix-producing interstitial myofibroblasts.<sup>20</sup> In addition to albumin, transferrin, and Ig, glomerular proteinuria result in ultra filtration of high molecular weight precursor forms of complexes of growth factor proteins such as insulin like growth factor 1, hepatocyte growth factor and TGF-  $\beta$ 1. Micropuncture studies in proteinuric rats and experiment in cultured proximal tubular cells allowed documentation that growth factor become activated in tubular fluid to interact with tubular cell receptors, thereby causing secretion of collagen types I and IV, MCP-1, and RANTES.<sup>20,22</sup> Chemokines upon secretion in the basolateral compartment of tubular cells, also may stimulate macrophages in the renal interstitium to secrete TGF- $\beta$ , which in turn is a powerful stimulus for the expression of extracellular matrix

protein by interstitial myofibroblasts. Besides proteins also stimulate apoptosis of tubular cells. The combined effect of apoptosis, upregulation of chemokines, oxidant stress and increased production of extracellular matrix protein results in renal damage.<sup>18-22</sup>

## Therapeutic and preventive strategies

### Blood Pressure

Three large trials have randomized kidney disease patients to two different levels of BP control, a usual goal (approximately 140/85 mmHg) or a low goal (approximately 125/75 mmHg), and observed the effects on proteinuria. The studies are the MDRD study, the Appropriate Blood Pressure Control in Diabetes (ABCD) study, and the African American Study of Kidney Disease and Hypertension (AASK). The low BP goal either reduced proteinuria by 50% or prevented the twofold to threefold increase in proteinuria observed in the usual BP goal patients. In the MDRD study, the greater the baseline proteinuria, the greater was the per cent reduction in proteinuria in the low goal group. Compared with the usual BP goal, the low BP goal reduced stroke rate and left ventricular mass index. Benefits of the low goal have also been confirmed in hypertensive non-kidney disease patients.<sup>23, 24</sup>

### Angiotensin enzyme inhibitor

ACEI, rather than ARB, is the initial choice because, although both ACEI and ARB are antiproteinuric and renal protective, it is unclear whether ARB are cardioprotective to the level of ACEI. The HOPE trial ( $n > 9000$  patients) showed that ramipril significantly reduced the composite endpoint of death, stroke, and myocardial infarction.<sup>25,26</sup> In the LIFE trial ( $n > 9000$  patients), the composite endpoint of death, stroke, and myocardial infarction was reduced significantly by losartan but of the individual components of the composite endpoint, only stroke was significantly reduced.<sup>27</sup> Furthermore, in the OPTIMAAL trial ( $n = 5477$  patients), which compared losartan to captopril in patients with acute myocardial infarction and heart failure, captopril

was numerically better than losartan in reducing death, the primary endpoint ( $P = 0.069$ ). In the captopril trial, the combined endpoint of death or ESRD was reduced significantly.<sup>25-28</sup>

### Angiotensin receptor blockers

ARB are recommended in ACEI-intolerant patients (cough, angioedema, or allergy). Also, ARB may raise serum potassium less than ACEI. ARB are antiproteinuric and renoprotective in the nephropathy of type II diabetes. The American Diabetes Association recommends ARB as first-line therapy in type II diabetic patients with nephropathy because no large-scale trials demonstrate efficacy of ACEI in this group. However ARB may not be cardioprotective to the level of ACEI and may be less antiproteinuric. Theoretically, ARB might be more cardioprotective than ACEI therapy in part because most myocardial AngII is formed by chymase, not ACE, and ACEI do not inhibit chymase. Myocardial chymase is in interstitial cells, mast cells, and bound to extracellular matrix. If ARB can efficiently penetrate myocardial interstitium, they should be more effective than ACEI in attenuating myocardial AngII effects such as myocardial hypertrophy and fibrosis. However, ARB are highly protein bound, which could affect tissue penetration.<sup>29,30</sup>

### Combination ACEI and ARB

There is now clear evidence that combination ACEI/ARB therapy is more antiproteinuric than ACEI or ARB alone. Also combination ACEI/ARB may be more renoprotective than either drug alone as demonstrated in a recent large-scale trial in nondiabetic kidney disease. Therefore, early deployment of combination ACEI/ARB therapy can be recommended. The optimum antiproteinuric strategy appears to be addition of ARB to maximum ACEI in those who fail to achieve their proteinuria goal on ACEI alone. The theoretical benefits of combination therapy include those of ACEI therapy (increased bradykinin, decreased aldosterone, decreased AngII levels) and those of ARB therapy (blockade of AngII produced by chymase, and

increased AT2 receptor activation, which may be vasodilatory, antiproliferative, and antifibrotic). Diuretic therapy may increase the renoprotective effects of combination therapy. Combination ACEI/ARB therapy might be particularly effective in those with the ACE gene DD genotype where resistance to ACEI may be present. The incidence of hyperkalemia in combination ACEI/ARB therapy in CKD is similar to that of ACEI alone, even when ACEI and ARB are given in maximum recommended doses.<sup>25, 31</sup>

### Lipid Lowering Drugs

Controlled clinical trials show an antiproteinuric effect of lipid-lowering therapy, particularly statins and niceritrol, a nicotinic acid derivative. The mechanisms may include decreasing oxidative stress and prevention of lipid-induced podocyte damage from decreased nitric oxide production. The maximum recommended statin dose may be the appropriate starting dose, based on the remarkable benefits and safety of 40 mg/d simvastatin in the MRC/BHF study. This study also suggested that there may not be a blood lipid threshold for cardiovascular benefit of statin therapy. Combining ACEI and statins may further reduce proteinuria. Note that fenofibrate increases serum creatinine by as much as 35% because of increased creatinine production. Pravastatin has been shown to further reduce proteinuria when added to ARBs in hypertensive patients. Its withdrawal abrogated the effect.<sup>32, 33</sup>

### Other Modalities

Though there is no RCTs to provide level 1 evidence but weight reduction (BMI < 30), low protein diet (0.7 gm/kg/day), smoking cessation, use of anti oxidants, low salt intake, addition of aldosterone antagonists, avoidance of strenuous exercise, avoidance of contraceptive pills and NSAIDs have all been found to be useful adjuncts to reduce proteinuria.<sup>25,34</sup>

### Conclusions

Proteinuria is an early marker of increased CV risk

and hence early measures to reduce this need to be exercised. Adequate blood pressure control, ACEI or ARBs or their combination along with statins should be exhibited early enough with a goal to reduce enormous morbidity and mortality risk to this population.

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