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Challenging the conventional wisdom on diabetic nephropathy: Is microalbuminuria the earliest event?

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ABSTRACT

Microalbuminuria, urine albumin-to-creatinine ratio >30 and less than <300 mg/g has widely been accepted as biomarker of early diabetic kidney disease (DKD). Based on absence of beta-2 microglobulinuria in earlier studies on microalbuminuria, its possible tubular origin has been dismissed. Microalbuminuria has been assumed as a marker of endothelial injury.

Low-molecular-weight (LMW) proteins are smaller than albumin and less restricted by the glomerular filtration barrier. Normal individuals excrete no >10 to 20 mg/day low-molecular-weight proteins in urine, as they are absorbed in the proximal tubules. Thus, LMW proteinuria, or non-albumin proteinuria (NAP) is a reliable marker of tubular dysfunction in patients without glomerular involvement.

In some cohorts of patients with diabetes (DM), tubular proteinuria preceded microalbuminuria, and similar to the findings reported by Han et al. in this issue of the journal, the tubular origin of these proteins was confirmed through correlations with the increased urinary concentrations of *N*-Acetyl-β-D-Glucosaminidase (NAG), a marker of tubular-lysosomal injury.

Observations show significant NAP in the urine of DM patients without microalbuminuria; and that NAP correlates with NAG. In many studies it appears that NAP often precedes microalbuminuria, and is likely not the earliest biomarker of diabetic kidney disease. The conclusion that microalbuminuria is a biomarker of glomerular endothelial injury and that it is the earliest finding in DKD requires a re-appraisal. Data increasingly suggest that in early stages of DKD, NAP precedes microalbuminuria, and that microalbuminuria itself may be a consequence of impaired tubular reabsorption.

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Since the introduction by Keen et al. of an immunoassay for the measurement of micro quantities of albumin in the urine; and demonstration of increased albumin excretion in the urine of patients with diabetes mellitus (DM) with elevated blood sugar levels; microalbuminuria, generally defined as urine albumin-to-creatinine ratio (UACR >30 and less than <300 mg/g) has widely been accepted as a biomarker of early kidney involvement in DM patients. Based on the absence of beta-2 microglobulinuria in the earlier studies on microalbuminuria the possible tubular origin of microalbuminuria has been dismissed, and it has been assumed as a marker of endothelial injury.[1,2] Although there is extensive literature on microalbuminuria as being predictive of increased cardiovascular morbidity both in DM and hypertension; the assumption that it reflects endothelial injury is not supported by strong evidence. Data that support microalbuminuria as a biomarker of endothelial damage rather than tubular injury are conflicting and have generated considerable controversy.[3]

Since the late 1980s there have been numerous studies looking for non-albumin proteins in DM patients without proteinuria; and many other non-albumin low molecular proteins have been proposed superior to albumin. These low molecular weight proteins include alpha-1-microglobulin, immunoglobulin light chains, retinol binding protein, beta-2 microglobulin (b2m), and others.[4–7] In some cohorts, tubular proteinuria preceded microalbuminuria,[4] and similar to the findings reported by Han et al. in this issue of the journal, the tubular origin of these proteins was confirmed through correlations with the increased concentrations of *N*-Acetyl-β-D-Glucosaminidase (NAG) in the urine.[8] NAG, a ~140 kDa protein, is really not a tubular protein, but a marker of tubular-lysosomal injury and the observed correlations between a whole family of low-molecular weight proteins are convincing as evidence of tubular origin.

The studies that evaluate the low-molecular-weight proteins cannot seem to agree on a single surrogate representative protein and therefore are difficult to compare. As some tubulo-interstitial diseases of the kidney, such as cadmium nephropathy are characterized by beta-2 microglobulinuria; many investigators attempted to test it as a biomarker of tubulopathy in DM. Perhaps not surprisingly, there is significant

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inconsistency among these studies, because b2m is difficult to measure accurately. It is very unstable in urine under normal conditions and accurate measurement requires careful control of temperature and pH of the urine in morning samples, and addition of small quantities of proteolytic enzyme inhibitors. Thus, it is not a reliable biomarker.[9] Absence of b2m in the urine of DM patients with microalbuminuria in the earlier studies may well have been a methodological artifact having mislead investigators on the tubular origin of micro quantities of albumin in the urine.

Low-molecular-weight proteins are smaller than albumin and are relatively less restricted by the glomerular filtration barrier. In healthy individuals, significant quantities of these proteins are estimated to be present in the glomerular ultrafiltrate, but only minute quantities appear in the urine, as they are endocytosed by the proximal tubule cells through the endocytic receptors megalin and cubilin.[10–12] The endocytic process in the proximal tubules is very efficient. As a result, normal individuals excrete no >10 to 20 mg/day low-molecular-weight proteins in the urine. Thus, low molecular weight proteinuria, or non-albumin proteinuria (NAP) is a reliable marker of tubular disease in patients without glomerular involvement. In patients with biopsy-proven tubulointerstitial diseases and tubular range proteinuria, along with low-molecular-weight proteins, significant quantities of albumin is often present as the tubules' capacity to endocytose the "normally filtered" proteins is impaired. In such cases urine albumin-to-creatinine ratio (UACR) to total protein-to-creatinine ratio (UPCR) reflects predominance of non-albumin proteins. The UACR/UPCR ratio of <0.40 was 88% sensitive and 99% specific for the diagnosis of primary tubulointerstitial disease.[13] Conversely, glomerular involvement disrupting podocyte integrity, i.e., podocytopathy, usually results in massive proteinuria comprising albumin and larger proteins far exceeding the quantity of low-molecular-weight (tubular) proteins as glomerular filtration barrier is impaired. It is hard to imagine conditions that impair the glomerular filtration barrier so subtly and precisely that only micro quantities of albumin leaks in the urine.

Even a completely healthy glomerulus allows small quantities albumin in the ultrafiltrate (glomerular sieving coefficient is generally estimated at ~0.0006), but these small concentrations of albumin can be processed by the endocytic apparatus of the proximal tubules as data clearly show that albumin, just like low-molecular weight proteins bind to the tandem megalin-cubilin endocytic receptor, which explains why normal people have significantly <30 mg/day albumin in the urine.[10] Additional direct evidence of tubular origin of microalbuminuria can be found in studies evaluating the effect of inhibiting tubular reabsorption of filtered proteins by infusion of L-arginine, a maneuver well-known to block tubular protein endocytosis. L-Arginine infusion in healthy volunteers and in DM patients clearly shows that blocking tubular reabsorption increases the excretion of albumin well within microalbuminuria ranges along with other low-molecular weight proteins.[14,15]

The observations in diverse populations with DM since the 1980s showing NAP in the urine of apparently non-proteinuric patients, and that NAP generally correlates with NAG. NAP also correlates with parameters of glycemic control leaving little doubt that it is time to reassess the microalbuminuria paradigm. It is highly doubtful that microalbuminuria even when present is a marker of glomerular endothelial injury, but more likely is a marker of tubulopathy. Furthermore, in most studies it appears that non-albumin proteinuria often precedes microalbuminuria, and it is likely not the earliest biomarker of diabetic kidney disease. The conclusion that tubular proteinuria is the earliest finding in patients with DM requires a re-appraisal of our understanding of the natural history of DM, and suggests that tubular involvement precedes glomerular injury. The clinical implications of this conclusion are not completely clear at this time. At a minimum looking for non-albumin proteins in the urine of patients with DM and not focusing solely on microalbuminuria is likely to enable clinicians to diagnose kidney involvement in DM at even earlier stages. This in turn, should result in implementation of clinical interventions to prevent overt kidney disease at earlier stages.

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