Abstract
Microalbuminuria represents a condition wherein the urinary albumin excretion is in the range of 30 mg/24 hrs -300 mg/24 hrs. Recently microalbuminuria is gaining attention as more than just a surrogate marker of kidney damage. Although apparently representing passage of a rather trace amount of protein excretion, this has great implication in terms of clinico-pathological association with diabetic nephropathy and other cardiovascular complications in subjects with or without diabetes. Early detection of microalbuminuria is considered to be the aim of clinicians in context to patient’s health perspective so as to change the treatment strategy accordingly. Therefore quite reasonably, understanding of exact patho-physiological significance of microalbuminuria and its prognostic impact in kidney diseases should be of immense help in providing better clinical outcome. To this end, an approach from the clinical biochemistry perspective might provide a better overview. The present commentary is an endeavor to address several relevant issues in this context for development of a better insight.

Preamble
A consistent positive relationship between urinary albumin excretion and adverse clinical conditions of kidney like diabetic nephropathy, hypertensive nephropathy, end-stage renal diseases and cardiovascular diseases in patients with or without diabetes is well-known. Normal reference range of excretion of urinary albumin is considered below 30 mg in 24 hours; microalbuminuria signifies the minor quantity of albumin ranging between 30 mg/24 hrs - 300 mg in 24 hours to pass out of renal filtration barrier to appear in urine. 1

Biochemistry
Albumin; the Versatile Molecule
Albumin, is a small globular non-glycosylated protein with a molecular mass of around 66.3 kDa. Its relative abundance along with its high solubility rendered it to be an effective carrier in circulation. Suitable pK value (4.6) owing to its charged surface makes it easily dissociable at physiological pH; this factor coupled with its colloidal nature has been contemplated in its significant role for maintenance of plasma oncotic pressure. 2 It also has significant nutritional value for the renal tubular cells 3 and its high thiol content endows it with great antioxidant prowess. This versatility probably explains the parsimonious approach for conserving this molecule by kidney.

Synthesis of Albumin and its Regulation
Daily 10-15 gm of albumin is synthesized predominantly in hepatocytes accounting for 10% of the total protein synthesis. Synthesis of albumin is constantly regulated by changes in interstitial colloid pressure. 2 Besides that, nutritional status and various pathophysiological conditions including hormonal influence like thyroxin, corticosteroids and insulin also affect albumin synthesis. 3 Synthetic capacity can be increased up to 2-2.7 fold of its normal level as per the requirement of individual. 4 Lower synthetic rate in diabetic subjects might be recovered with insulin infusion. 3 Conversely, as major negative acute phase reactant there is plummet in its level during pro-inflammatory state. 2 Albumin escapes from plasma at rate 4.5% / hr and from vascular space through lymphatics at same rate by its absorption. Interestingly, albumin synthesis is very much linked and governed by its fractional catabolic rate, may be the most significant fact during dietary protein restricted state or at the deficient synthetic state. 2,4 The catabolic fate of albumin is determined mostly through utilization by vascular endothelium (almost 90%) and the rest is by renal tubular epithelial cells. 5 Therefore, this intricate and apparently paradoxical relationship possibly suggests a subtle balanced nexus between catabolic and anabolic processes.
Renal Membrane and Filtration of Albumin

Glomerular filtration is considered to result in formation of an essentially albumin-free filtrate due to remarkable sieving through a serially arranged fenestrated endothelium with its glycocalyx, the glomerular basement membrane and podocyte slit diaphragm that almost completely prevents large and negatively charged macromolecules from reaching Bowman’s capsule.6 However, both logical analysis as well as supportive evidences refute substantial impact of the later process as a determinant of urinary albumin excretion. Classically, the proposed mechanism for rigorous restriction of albumin passage is based on the exclusion of albumin from the glomerular capillary wall through size selectivity and probably more owing to charge repulsion; which would be further clarified in the context of the clinical correlates described later. As opposed to estimated glomerular sieving coefficient (GSC) of 0.02 to 0.1 by inert polysaccharide probes with a similar hydrodynamic radius like albumin (36 Å), the GSC for albumin determined by micro-puncture was found to be markedly low at the level of 0.0006 to allow free passage. However, renal filter matrix trickle albumin only in trace amount. The hypothesis put forward to account for this apparent paradox is the charge selectivity, whereby negatively charged albumin is repelled by a theoretical, fixed, negative charge of the glomerular capillary wall at physiological pH.8 Endothelial cells express negatively charged glycoprotein capsule containing sialic acid residues.9 The basement membrane, which is a porous matrix of negatively charged proteins including type-4 collagen, laminin, and proteoglycans (agrin and perlecan) and fibronectin further forms an important filtration barrier to plasma proteins.6,10

Besides filtration barrier, another plausible checkpoint of albumin release in urine is through a catabolic mechanism for albumin degradation through megalin-cubulin complex11 to conserve the amino acids rather than a full-fledged retrieval process. The process is mostly completed by proximal convoluted tubule followed by its degradation in the luminal cells. These forms basis of the debate between two hypotheses: albumin uptake deficiency versus filtration barrier defect. If considered to be significant, albumin retrieval premise which have been revived due to the observed higher GSC for albumin in animal experiments essentially need to be substantiated with commensurate albumin uptake along with its delivery to the circulation. However because of its size, albumin cannot leave the tubular lumen on the paracellular route across the tight junctions. Furthermore, following this hypothesis, albumin is not cleaved in the tubular lumen and therefore does not cross the apical membrane of the proximal tubular cell in the form of free amino acids.12

Thus the possible mechanism is shown to mediate albumin reabsorption through endocytosis, followed by the proposed mechanism of transcytosis for its passage to circulation.9,12 However certain technical limitations coupled with the lack of sufficient evidences from human experiments keep this question to be resolved yet.

In pathological conditions e.g. massive proteinuria in nephrotic syndrome, probably results from marked reduction in the charge-selective properties of the filtration barrier and also alteration in the pore size in the slit diaphragms formed by inter-digitating foot processes of adjacent podocytes (epithelial cells).8 However, in context of glomerulonephritis cases, a higher probability of loss of charge factor seemed to be operative with lesser extent of the pathology, whereas relative higher edge of alteration of pore size was contemplated in more severe condition.10

Clinical Chemistry

Rationale

Analysis of healthy individuals’ urine shows that only little albumin (up to 30 mg/ day) undergo excretion.1 Although kidney is a major portal of excretion, the passage of any substance in urine may not necessarily indicates renal damage but actually display a mere reflection of certain systemic disorders like metabolic diseases, with or without renal pathology. Therefore, albumin in urine is considered not just as a surrogate marker of renal damage but also as a reflection of various systemic pathophysiology.1 Given the great physiological significance of albumin, teleologically special strategy has been devised to prevent its loss; thus traces of albumin in urine may actually be considered as a mark of deviation from physiological homeostasis.

In healthy subjects, filtration and reabsorption of albumin are in equilibrium in which <1% of filtered albumin reaches finally in the urine. Pathophysiological states with increased renal albumin excretion, the so-called albuminuria or more generalized proteinuria therefore may be explained by any of the three basic mechanisms currently available: 1) glomerular injury resulting in excess filtration of protein e.g nephrotic syndrome; 2) tubular injury resulting in excessive production and excretion of tubular proteins (e.g., Tamm-Horsfall protein); and 3) over-excretion of plasma proteins due to high plasma concentration, e.g., in multiple myeloma.

Diagnostic Dilemma-albuminuria versus Microalbuminuria

Early detection of
microalbuminuria as a risk prediction marker demands high sensitivity test. The term “proteinuria” refers to increased urinary excretion of any protein without any specific type; “albuminuria” refers specifically to increased urinary excretion of albumin only. “Microalbuminuria” specifically refers to albumin excretion above the normal range but below the level of detection by the conventional tests for total protein. In fact since such routine tests fail to detect very early stage proteinuria (albuminuria, to be precise) therefore this so called term ‘microalbuminuria’ came into existence, which might be confusing to represent incorrectly the excretion of certain minor version of albumin in terms of size and not the factual lower rate of excretion.

Popularly known dry chemistry based dipstick methods although prove to be quite sensitive test however lack desirable lower detection threshold. For regular screening of albuminuria in random sample, certain improvised rapid format semi-quantitative dip-stick method with advantage of reasonably good sensitivity and specificity may be considered. On the other hand, lack of ability for exact quantification, variation with diluted or concentrated sample coupled with the need of confirmation of specific amount of albumin for proper assessment of damage and consequent prognostic impact, limits its use. Hence owing to technical difficulty of conventional estimation approach coupled with its clinical significance, it is imperative to consider higher resolution immune-chemical techniques. Recent addition of HPLC method in the diagnostic repertoire may provide higher sensitivity.

Guidelines for detection and monitoring of proteinuria in adults and children also differ because of differences in the prevalence and type of chronic kidney disease. Large intra-individual and diurnal variation along with lack of available data about any contributing biological factors may also account for lack of precision in available lab results. Furthermore, there is inconsistency between laboratories regarding sample type, units of reporting, and reference intervals or cut points. Recent guideline should bring in consensus to address these issues for setting the precise recommendations to define urine sampling technique, detection method for albuminuria, and a specific upper and lower limit for microalbuminuria suitable for diabetes, hypertension, and cardiovascular disorders.

Therefore off late, approximate estimation of excretion of urine albumin in mg/ gm of creatinine is routinely followed. For measurement of the ratio between albumin to creatinine, test can be done with spot collection of urine sample allowing simultaneous assessment of albumin against creatinine for a projected effect of renal clearance; therefore, it gives an indication of total albumin excretion in 24 hours being unaffected by the variation in concentration. The cut-off urine albumin to creatinine ratio for microalbuminuria are: ≥2.5 mg/mmol of creatinine and ≥2.5 mg/mmol creatinine in females and males respectively. As per The National Kidney Foundation guidelines screening for microalbuminuria is needed for in all patients with diabetes, hypertension, family history of chronic kidney disease with age above 60 years, and for certain racial and ethnic minorities.

Clinico-pathological Correlate

Diabetic Nephropathy and Cardio-metabolic Consequences

Diabetes mellitus appears the most common cause of end-stage renal diseases. Approximately 50% of diabetes type-1 individuals within 10 years and in >75% by 20 years develop in diabetic nephropathy and 20-40% of type-2 diabetes patients eventually may develop in to end-stage renal diseases. Glycative change associated loss of charge of filtration membrane and binding of advanced glycated end product (AGE) on podocyte receptor which are selectively expressed in glomerular epithelial cells may lead to increased glomerular permeability with resultant microalbuminuria in Diabetes mellitus. Microalbuminuria is generally first clinical sign of such renal dysfunction in diabetes. Development of diabetic nephropathy is characterized by progressive increase in protein excretion, particularly albumin, with early rise in systemic blood pressure and late decline in glomerular filtration. Passage of protein molecules through kidney may prove detrimental to the ultra-structure of the renal tissue giving rise to perpetual damage and ultimately may culminate towards total renal failure. Hence, American Diabetes Association (ADA) recommends screening for microalbuminuria annually for patients with type 1 diabetes of more than 5 years duration and for patients with type 2 diabetes from the first time of diagnosis as a prognostic indicator for development of CVD. Screening for diabetic nephropathy involves monitoring for at least yearly for urinary albumin excretion >30 mg per day. However, confounding factors should be considered in case of such a marker like microalbuminuria due to the obvious reason of its association with a wide spectrum of pathology. For example factors like hyperinsulinemia and associated insulin resistance, polymorphism of the D allele of ACE gene might be associated with higher prevalence of microalbuminuria. One should
also consider the baseline excretion of urinary albumin of a subject. In subjects with hypertension, antihypertensive drugs also can change the course of developing microalbuminuria.\textsuperscript{19} Besides these, any suspected aetiology of vascular pathology including smoking habit\textsuperscript{20} might have possible impact on such process. So they need to be considered before diagnosis of the diabetic nephropathy using this marker.

Both glycemic control (HbA\textsubscript{1c} <7\%) and blood pressure control (<130/80 mm of Hg) especially after the onset of renal damage is recommended as per guidelines of ADA,\textsuperscript{15} presumably because of hypertension induced glomerular damage as reported earlier from both animal as well as in human studies.\textsuperscript{21}

Vasculature and renal glomerulus have a lot in common structurally and functionally.\textsuperscript{22} According to Steno hypothesis, albuminuria might reflect a general vascular dysfunction and associated leakage of other plasma molecules such as low density lipoproteins into the vessel wall that may lead to inflammatory response which in turn perpetuate atherosclerotic pathology.\textsuperscript{22} Consequently the ADA recommendation for LDL level to be maintained below 100 mg/dl for the patients with renal disease is a justified proposition to prevent any such vascular condition to be superimposed.\textsuperscript{15} Many studies concluded that microalbuminuria can be an independent risk factor for all cause and cardiovascular mortality and coronary vascular events within groups of patients with diabetes or hypertension and also in general population.\textsuperscript{23}

The hallmark of cardiovascular diseases in diabetes is accelerated atherosclerosis of medium and large-sized arteries might be contributed by multiple factors like binding of advanced glycated end products on receptors of endothelial cells of glomerulus and generation of various growth factors and cytokines favoring pro-inflammatory milieu to accentuate such process.\textsuperscript{24} Moreover, diabetes associated changes in blood viscosity might lead to hypertensive features.\textsuperscript{24} Reportedly, a positive co-relation between high blood pressure and microalbuminuria exists; hypertension contributes to renal dysfunction by increasing glomerular vascular pressure and capillary damage along with possible tubular defect.\textsuperscript{21} The mesangial cell proliferation and related matrix modification in diabetes and hypertension associated induction of angiotensin-2 is also responsible for the loss of renoprotective effect.\textsuperscript{25} Additionally, diabetic nephropathy associated high level of glucose in the filtrate gets reabsorbed along with excess sodium in the renal tubule that can enhance local angiotensin\textsuperscript{26} with a feasible indirect effect on atrial natriuretic peptide release. It is well established that there is strong correlation between degree of hypertension and the rate of progression of overt diabetic nephropathy in type-1 as well as type-2 diabetes mellitus.\textsuperscript{27} In corroboration with this view, the successful use of ACE inhibitor, which decrease such pressure are actually proved to be beneficial. An earlier Study\textsuperscript{28} demonstrated that use of ACE inhibitors delay progression of less severe selective albuminuria to severe non-selective proteinuria; further combination of ACE inhibitors with angiotensin receptor antagonist have intensified blood pressure control to the kidney pathology.\textsuperscript{29} These proofs are enough for establishing the key pathogenic role of vascular effect on kidney damage which is mirrored by microalbuminuria as a tell-tale evidence of such pathology. Overall this pathology may be translated into accentuation of renal damage leading to deteriorating renal function. Hence alteration in albumin excretion should be considered not merely as a surrogate marker but as an important risk predictor for progressing cardio-metabolic disorders leading cardiovascular events. Therefore, quite obviously, microalbuminuria is gaining attention as a predictive as well as prognostic marker of atherogenesis. Moreover the excretion of this protein have noteworthy damaging potential\textsuperscript{30} which opens up a new perspective and makes us stand before an intriguing dilemma of cause and effect relationship between vascular pathology and such protein excretion.

Therefore all patients with microalbuminuria (except which are self-limiting) should be screened for macro-vascular diseases and if detected, aggressive interventions should be taken to reduce cardio-metabolic complications.

The Overall Impact and Future Prospect

With the above mentioned perspective, prognostic importance of microalbuminuria for predicting microvascular complications and also its progression towards imminent macrovascular pathologic stage leading to serious fallout of diabetic nephropathy and cardiovascular diseases\textsuperscript{21,25,28} might be well appreciated. However, due to impact of biological and analytical variations, microalbuminuria as a prognostic parameter although quite significant but may not be sufficient alone to predict the progress of renal damage.\textsuperscript{31} On the other hand, based on the evidence it might not be unjustified to extrapolate its importance as a risk predictor in several other diseases with common association of vascular pathology. Excretion of albumin in ‘microalbuminuric’ range is definitely an ominous sign which should be checked for with great degree of suspicion through standardized technique having reasonably good resolution and also need to interpret the result with justified clinical acumen to ensure timely intervention to
regress the pathology. It might be quite tempting to speculate its certain direct pathognomonic role beyond its present status as a mere risk predictor. This would definitely uplift its status as a versatile marker of vascular pathology.

References

2. Evans TW. Review article: albumin as a drug—biological effects of albumin unrelated to oncotnic pressure. Aliment Pharmacol Ther 2002; 16(suppl 5):6-11.
22. de Zeeuw D, Parving H, Henning RH. Microalbuminuria as an Early Marker for Cardiovascular Disease. JASN 2006; 17:2100-2105.