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TOPIC HIGHLIGHT

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Renal function in diabetic nephropathy

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Abstract

Diabetic nephropathy is the kidney disease that occurs as a result of diabetes. Cardiovascular and renal complications share common risk factors such as blood pressure, blood lipids, and glycemic control. Thus, chronic kidney disease may predict cardiovascular disease in the general population. The impact of diabetes on renal impairment changes with increasing age. Serum markers of glomerular filtration rate and microalbuminuria identify renal impairment in different segments of the diabetic population, indicating that serum markers as well as microalbuminuria tests should be used in screening for nephropathy in diabetic older people. The American Diabetes Association and the National Institutes of Health recommend Estimated glomerular filtration rate (eGFR) calculated from serum creatinine at least once a year in all people with diabetes for detection of kidney dysfunction. eGFR remains an independent and significant predictor after adjustment for conventional risk factors including age, sex, duration of diabetes, smoking, obesity, blood pressure, and glycemic and lipid control, as well as presence of diabetic retinopathy. Cystatin-C (Cys C) may in future be the preferred marker of diabetic nephropathy due differences in measurements of serum creatinine by various methods. The appropriate reference limit for Cys C in geriatric clinical practice must be defined by further research. Various studies have shown the importance of measurement of albuminuria, eGFR, serum creatinine and hemoglobin level to further enhance the prediction of end stage renal disease.

Key words: Chronic kidney disease; End stage renal disease; Glomerular filtration rate; Estimated glomerular filtration rate; Microalbumin; Cockcroft-Gault formula; Modification of diet; Renal disease; Cystatin-C

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Type 2 diabetes mellitus has quickly become a global health problem due to rapidly increasing population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity. There is, therefore, an urgent need to prevent diabetes and its complications. Diabetes is the major cause of end-stage renal disease (ESRD) both in the U.S. and around the world and has enormous medical, social and economic consequences. Diabetes affects the kidney in stages. At the onset of diabetes, the kidney grows large and the glomerular filtration rate (GFR) becomes disturbed. Most recent basic and clinical research has pointed toward sclerosis and kidney failure. The morbidity and mortality

caused by diabetes mellitus can be reduced by regular screening, early detection, and appropriate treatment of chronic complications. Thus, this discussion will focus on development of kidney damage, the various markers available and approaches to development of future markers to enhance the detection of ESRD at the earliest possible stage.

EPIDEMIOLOGY OF RENAL FAILURE

The global prevalence of diabetes is expected to increase from 4% in 1995 to 5.4% by the year 2025[1]. Currently, the countries with the largest number of diabetic patients are India, China and United States. The acute and chronic complications of diabetes mellitus are major causes of hospital admissions. Asian patients have shown evidence of macro and micro vascular disease at the time of diagnosis of diabetes when compared to Europeans[2].

Diabetes is the most common cause of kidney failure, accounting for nearly 44 percent of new cases[3]. Even when diabetes is controlled, the disease can lead to chronic kidney disease (CKD) and kidney failure. Kidney failure is the final stage of chronic kidney disease. Nearly 24 million people in the United States have diabetes and nearly 180 000 people are living with kidney failure as a result of diabetes[4]. The prevalence of nephropathy in India was less (8.9% in Vellore, 5.5% in Chennai) when compared with the prevalence of 22.3% in Asian Indians in the UK[5]. In chronic renal failure patients the prevalence of diabetic nephropathy was 30.3% followed by chronic interstitial nephritis (23%) and chronic glomerulonephritis (17.7%)[6].

African Americans, American Indians, and Hispanics or Latinos develop diabetes, CKD, and kidney failure at rates higher than Caucasians. Scientists have not been able to explain these higher rates and the interplay of various risk factors.

RISK FACTORS FOR DEVELOPMENT OF DIABETIC COMPLICATIONS

Diabetic nephropathy is the kidney disease that occurs as a result of diabetes. Nephropathy is the leading cause of chronic renal failure worldwide and is responsible for renal failure in about one third of patients who undergo dialysis. It is suggested that patients with common risk factors including greater duration of diabetes, hypertension, poor metabolic control, smoking, obesity and hyperlipidemia are more prone to develop diabetic complications[7]. In a retrospective study done by Klag *et al*[8] it was found that elevations of blood pressure are a strong independent risk factor for end-stage renal disease and that interventions to prevent the disease need to emphasize the prevention and control of both high-normal and high blood pressure.

The "Asian Indian Phenotype" refers to certain unique clinical and biochemical abnormalities in Indians which include increased insulin resistance, higher waist circumference despite lower body mass index, lower adiponectin and higher C-reactive protein levels. This phenotype makes Asians more prone to diabetes and premature complications. Asians show a trend towards higher systolic and diastolic blood pressures, possibly due to more patients with nephropathy, although this is not significantly different. Total cholesterol and LDL cholesterol levels were very similar between Europeans, Americans and Asians, but HDL cholesterol was significantly lower and triglycerides level was significantly higher in Asian patients[9].

James Sowers *et al* reviewed aspects of the association of diabetes with renal disease, emphasizing that CKD and albuminuria are associated with increased rates of cardiovascular disease (CVD) and mortality[10], and should be considered part of the cardiovascular risk factors in persons with diabetes. Lorenzo *et al*[11], found that the development of glomerular filtration rate < 60 mL/min per 1.73 m^2 was associated with increased fasting insulin, triglycerides, free fatty acids, and uric acid and also with antihypertensive treatment, although not with waist circumference, controlling for age, sex, ethnicity, blood pressure, glucose, and C-reactive protein in nondiabetic persons. This points towards the association of CKD with the risk of development of diabetes.

PATHOGENESIS OF KIDNEY DAMAGE

There are various mechanisms of albuminuria which involve abnormalities of the glomerular endothelial barrier[12], causing excessive filtration as well as reduction of renal tubular cell albumin degradation and reabsorption. Glomerular hypertension, inflammation, and oxidative stress worsen albuminuria, with angiotensin-Ⅱ[13] and mechanical stress factors contributing to these processes.

Renal disease in diabetes is found to be associated with abnormalities of vasodilatation and generates reactive oxygen species mediated by endothelial derived nitric oxide (NO), suggesting linkage between vascular and metabolic abnormalities. Angiotensin Ⅱ and aldosterone, interacting with pulse pressure and increased systolic blood pressure, activate NADP oxidase, which acts as mediator of oxidative stress. Angiotensin Ⅱ increases metabolism of NO to peroxynitrite[13], which further impairs endothelial-derived vasodilation.

In another mechanism, decrease in the ability to produce endothelial progenitor cells (EPCs), which can be quantitated with the cellular marker CD 34, leads to increased CVD risk. These cells derived from bone marrow, play a role in replacing damaged endothelium and are reduced in people with decreased endothelium-dependent vasodilation[14]. Both angiotensin Ⅱ and aldosterone inhibit production of EPCs, while angiotensin converting enzyme inhibitors (ACEIs) increase their levels.

Sowers *et al*[15] discussed the relationship between dyslipidemia and CKD, hypothesizing that the mechanism

Table 1 The various tests for chronic tubular dysfunction in diabetic nephropathy

Test name	Use
Blood urea nitrogen (serum or plasma) Method: Spectrophotometry	Initial diagnosis of acute or chronic kidney disease
Creatinine (serum or plasma) Method: Spectrophotometry	Initial diagnosis of acute or chronic kidney disease
Microalbumin (urine) Method: Immunoturbidimetric	May be used as a screening test Useful in diabetic patients to assess baseline renal function Useful in monitoring diabetic nephropathy in insulin-dependent diabetes mellitus
Creatinine based glomerular filtration rate (estimated) Method: Spectrophotometry	Estimate renal function and use as monitoring tool (Test reports serum creatinine reference intervals)
Cystatin-C based glomerular filtration rate (estimated) Method: Nephelometry	May be useful sensitive marker of renal disease; however, test lacks specificity due to reference range inavailability
Retinol-binding protein 4 (RBP4) Method: Non-commercial enzyme-linked immunosorbant assay (ELISA)	May be used as a marker for early diabetic nephropathy. Limited studies are available
Adiponectin Method: Competitive radioimmunoassay	Shown inverse correlation with renal dysfunction in type 2 diabetes
Connective tissue growth factor (CTGF) Method: ELISA	CTGF excretion is correlated inversely with GFRs
Alpha-1-microglobulin (urine) Method: Nephelometry	May indicate renal involvement in diabetic patients
Liver type fatty acid binding protein (L-FABP) Method: ELISA	Expressed in proximal tubular cells and may associated with severity of diabetic nephropathy. Larger conclusive studies are required

of action of statins is an increase in endothelial NO synthase transcription *via* the phosphatidylinositol 3-kinase (PI3K) pathway and statin-induced inhibition of the mobilization of small molecular weight G-proteins. Also, Sowers suggested that the direct renin inhibitor aliskiren has similar benefits in renal disease to those of angiotensin receptor binders.

Charles Heilig *et al*[16] discussed the relationship of renal glucose transporter expression in relation to the development of diabetic nephropathy. Expression of GLUT1, the major mesangial glucose transporter, regulates extracellular matrix production. Mesangial cells overexpressing GLUT1 show increased production of both types Ⅰ and Ⅳ collagen, as well as increased fibronectin and laminin production, leading to a phenotype similar to that of diabetes. In animal models, GLUT1 overexpression in glomeruli creates a nephropathy phenotype resembling that of diabetic renal disease with increased mean glomerular volume, mesangial expansion, and sclerosis.

DEVELOPEMENT OF KIDNEY DISEASE

Diabetic nephropathy takes many years to develop. In some people, the filtering function of the kidneys is actually higher than normal in the first few years of their diabetes.

In people who are developing kidney disease small amounts of the blood protein albumin begin to leak into the urine, a condition called microalbuminuria. The kidney's filtration function usually remains normal during this period. As the disease progresses further, more albumin leaks into the urine, a stage known as macroalbuminuria or proteinuria. As the amount of albumin in the urine increases, the kidney's filtering function usually begins to drop, resulting in the body's retention of various wastes. As kidney damage develops, blood pressure also often rises or hypertension may attenuate the process of renal injury. Early detection of renal damage may help to delay the process.

As these processes are slow, kidney damage rarely occurs in the first 10 years of diabetes, and 15 to 25 years will usually pass before kidney failure occurs. For people who live with diabetes for more than 25 years without any signs of kidney failure, the risk of ever developing it decreases.

BIOMARKERS OF CHRONIC TUBULAR DYSFUNCTION

The early detection of diabetic nephropathy, resulting in timely intervention with particular attention to blood pressure control (thus limiting proteinuria), glycemic control, smoking cessation, and accentuation of cardiovascular risk, can improve long-term outcomes and retard progression to ESRD.

Tubular proteinuria results when glomerular function is normal but the proximal tubules have diminished absorbing capacity. The biomarkers of this process are as Table 1.

Blood urea nitrogen

Blood tests for Blood urea nitrogen (BUN) and creatinine are the simplest way to monitor kidney function. These substances are normal metabolic waste products that are excreted by the kidneys. Urea is a byproduct of protein breakdown. A test can be done to measure the amount of urea nitrogen in the blood. In kidney disease, these substances (as well as numerous others) are not excreted normally, and so they accumulate in the body thus causing an increase in blood levels of urea. The normal level of BUN is 7-20 mg/dL[17].

Creatinine

Serum creatinine is primarily a metabolite of creatine, almost all of which is located in skeletal muscle. The normal level of creatinine is 0.8 to 1.4 mg/dL. Females usually have a lower creatinine (0.6 to 1.2 mg/dL) than males, because they usually have less muscle mass[17].

The amount of creatine per unit of skeletal muscle mass is consistent and the breakdown rate of creatine is also consistent. Thus, plasma creatinine concentration

is very stable and a direct reflection of skeletal muscle mass[18]. Interestingly, Nobuko Harita *et al*[19], hypothesized that, lower serum creatinine is associated with an increased risk of type 2 diabetes, which might reflect a lower volume of skeletal muscle. Skeletal muscle is a major target tissue of insulin and a lower volume of skeletal muscle would mean fewer target sites for insulin which causes increase in insulin resistance. This leads to the development of type 2 diabetes[20]. This may explain in part the pathogenesis of type 2 diabetes associated with lower serum creatinine.

Creatinine based GFR

GFR is the best measure of kidney function since it accounts for age, BMI and sex. GFR measures the rate at which the kidneys' two million glomeruli filter plasma in order to process it and remove waste products from it. If the kidneys are injured by chronic kidney disease, the GFR gradually declines, and the amount of remaining kidney function can be estimated by measuring or calculating the GFR. The normal value for GFR in a normal-sized person is 100-150 mL/min.

Currently the two most common methods for determining GFR are creatinine clearance and estimated GFR (eGFR).

Creatinine clearance: Creatinine clearance requires a 24 h urine collection. A blood sample is drawn at some point during the 24 h period, and creatinine clearance, can then be calculated. However, because a small amount of creatinine is released by the filtering tubes in the kidneys, creatinine clearance is not exactly the same as the GFR. In fact, creatinine clearance usually overestimates the GFR, particularly in patients with advanced kidney failure. Normal clearance values are: Male: 97 to 137 mL/min; Female: 88 to 128 mL/min[21].

There are several factors that may interfere with the accuracy of the test. These include: (1) Incomplete urine collection; (2) Pregnancy; and (3) Vigorous exercise. Creatinine clearance measurements can also be affected by drugs, such as: cimetidine, trimethoprim, and drugs that can damage the kidneys (cephalosporins).

The creatinine clearance test should only be done for patients who are medically stable. Other patients may have a rapidly changing creatinine clearance, and therefore any result may be inaccurate.

eGFR: Formula-derived eGFR results have become widely used in clinical practice. The National Service Framework for Renal Services in the U.K. recommends the adoption of formula-derived eGFR in the annual evaluation of all patients with diabetes[22]. It is anticipated that this process will aid early identification and therefore improve longterm outcomes for those with diabetic nephropathy.

The American Diabetes Association recommends estimation of glomerular filtration rate by eGFR (in millilitres per min per 1.73 m^2), which is calculated by the Cockcroft-Gault (CG) formula[23], corrected for Body Surface Area (BSA), and the Modification of Diet in Renal Disease (MDRD)[24] equation in all patients with diabetes. (1) CG derived eGFR: A simpler method for estimating creatinine clearance is based upon a formula (the Cockcroft-Gault formula) that includes a person's age, gender, weight, and serum creatinine level, but does not require the collection of a 24 h urine sample. The CG formula is[23] as follows: $eGFR = [140 - age\ (years)] \times weight\ (kg) \times k \times c / serum\ creatinine\ (\mu mol/L)$, (k is 1.23 for men and 1.04 for women and c adjusts for BSA[25]. c = 1.73/BSA, with BSA calculated using the following DuBois[26] formula), {BSA (m^2) = [weight (kg)] 0.425 × [height (cm)] 0.725 × 0.007184}; (2) MDRD derived eGFR: Renal function can be assessed by serum creatinine and eGFR and calculated using the abbreviated MDRD formula as follows[24]: $eGFR = 186 \times (SCR \times 0.011)^{-1.154} \times (age)^{-0.203} \times (0.742,\ if\ female) \times (1.210\ if\ African\ American)$ (SCR was serum creatinine expressed as μmol/L). Renal function has been graded according to the Kidney Disease Outcomes Quality Initiative guidelines: stage 1, ⩾ 90 mL/min per 1.73 m^2; stage 2, 60-89 mL/min per 1.73 m^2; stage 3, 30-59 mL/min per 1.73 m^2; stage 4, 15-29 mL/min per 1.73 m^2; and stage 5, < 15 mL/min per 1.73 m^2 (gf4-13); (3) Reexpressed MDRD equation: As significant error is introduced in the MDRD equation by use of different creatinine assays or calibration methods, the simplified MDRD was recently recalculated with serum creatinine measurements calibrated to an enzymatic assay[27]: $eGFR = 175 \times [serum\ creatinine\ (mg/dL)]^{-1.154} \times (years)^{-0.203} \times 0.742\ (if\ female) \times 1.212\ (if\ African\ American)$; (4) MCQ equation: A new equation was developed by Rule *et al*[28] for GFR estimation in chronic kidney disease patients and in healthy persons for the diagnosis of chronic kidney disease. This is expressed as: $(1.911 + 5.249/SCr - 2.114/SCr^2) - [0.00686 \times age\ (years)] - 0.205$ if female, where SCr is serum creatinine [in milligrams per deciliter]; and (5) Considerations: eGFR is used for assessment of kidney function in patients with diabetes. However, despite validation in chronic kidney disease, eGFR has limitations in patients with preserved kidney function. These equations do have recognized limitations, including a tendency to significantly underestimate higher levels of GFR[29]. Additionally, Parving and colleagues[30] demonstrated that in type 2 diabetic subjects with macroalbuminuria, eGFR had a poor sensitivity for GFR values < 60 mL/min per 1.73 m^2. In obese patients with established kidney disease, the Cockcroft-Gault equation overestimates GFR while underestimating GFR in lean subjects, possibly due to increasing weight; while performance of the MDRD equation in such patients is consistent regardless of weight. Bias of the Cockcroft-Gault formula was most pronounced in lean subjects, diminishing with increasing body weight. Conversely, bias of the MDRD equation increased with increasing body weight[31]. In obese patients, excess body weight is mainly adipose

tissue, whereas creatinine is primarily generated by muscle. In the Cockcroft-Gault equation, body weight is proportional to GFR; therefore, increasing body weight without a proportional increase in creatinine generation will tend to increase the estimation of GFR. However, weight is not included in the MDRD equation and therefore cannot influence performance.

There are reports[32,33] that variation in calibration of the creatinine assay has an adverse impact on the performance of eGFR to estimate GFR, particularly at low levels of serum creatinine.

Cystatin-C based eGFR

A large percentage of individuals with type 2 diabetes pass through a period of pre-diabetes and may experience early renal dysfunction, e.g. a GFR > 60 mL/min per 1.73 m^2. Serum creatinine has been found to be defecient to detect mild renal impairment, even when used with prediction equations[34,35]. So, interest has developed in Cystatin-C, a non-glycosylated basic protein, as a potential endogenous filtration marker of GFR. Cystatin-C is a cysteine protease inhibitor that is produced by virtually all nucleated cells and released into the bloodstream. It is entirely filtered by the kidney glomerulus and metabolized by the proximal tubule[36].

Various formulae have been used to measure serum cystatin levels by different methodologies. Recent estimations were done using a particle-enhanced immunonephelometric[37] assay or immunoturbidimetric assays. In all formulae, Cys C is serum cystatin-C (in milligrams per liter).

Arnal *et al*[38] estimated eGFR in 208 patients aged 1-80 years with various etiologies as follows: Cys-eGFR (Arnal-Dade) = 74.835/(Cys C1.333). Rule *et al*[39] studied native kidney disease (gf7-15) patients (n = 204) having hypertension as suspected etiology: Cys-eGFR (Rule) = 66.8 - (Cys C) - 1.30. Isotopic GFR (iGFR) was measured by iothalamate clearance. MacIsaac *et al*[40] studied 126 diabetic patients (mainly type 2 diabetes). The iGFR was measured by clearance of 99mTc-diethylene-triamine-penta-acetic acid. The equation is as follows: Cys-eGFR (MacIsaac) = (84.6/Cys C) - 3.2. Tan *et al*[41] used an unbiased conversion algorithm between plasma cystatin-C and iGFR measured by iohexol clearance in type 1 diabetes, including a subgroup of healthy subjects as follows: Cys-eGFR (Tan) = (87.1/plasma Cys C) - 6.87. Erosha *et al*[37] measured GFR using the equation Cys-GFR (Erosha) = (86.7/Cys C) - 4.2. The intra- and interassay coefficient of variation for Cystatin-C were 2.58 and 3.95%, respectively, at a concentration of 1.54 mg/L.

Considerations: It has been shown that Cystatin-C is a more sensitive indicator of mild renal impairment and may better estimate the GFR than serum creatinine[42]. Moreover, concentrations of Cystatin-C are not affected by sex, age, or muscle mass[43]. There is supportive evidence that the reciprocal of Cystatin-C correlates more closely with isotopic GFR than the CG or MDRD equations in subjects with mild renal impairment[44]. As the identification of those with pre-diabetes is assuming greater importance, Cystatin-C may play a role in detection of the association between renal and heart disease in etiology of pre-diabetics. Recent clinical trials[45,46] among people with pre-diabetes have provided convincing evidence that early intervention can significantly delay or prevent the progression to type 2 diabetes. However, concerns remain regarding intrapatient variation and the effect of certain drugs and hormonal levels on Cystatin-C concentration[47].

Microalbumin (urine)

Albuminuria is a well-known predictor of poor renal outcomes in patients with type 2 diabetes and in essential hypertension[48,49]. Albuminuria has also been shown to be a predictor of cardiovascular outcomes in these populations. There is emerging data that reduction of albuminuria leads to reduced risk of adverse renal and cardiovascular events[50,51]. It has become increasingly clear that albuminuria should not only be measured in all patients with type 2 diabetes and hypertension, but also that steps should be taken to suppress albuminuria in order to prevent future renal and cardiovascular adverse events. Albuminuria may reflect underlying renal expression of vascular damage, hypertension, endothelial dysfunction[12], and low-grade inflammation[52].

Expected results: Microalbuminuria is defined as levels of albumin ranging from 30 to 300 mg in a 24 h urine collection[53]. Overt albuminuria, macroalbuminuria, or proteinuria is defined as a urinary albumin excretion of > 300 mg/24 h. Urinary albuminuria comprises 20%-70% or urinary total protein excretion. Albuminuria can be measured in several ways (Table 2): (1) measurement of albumin-to creatinine ratio in a random or first morning spot collection; (2) 24 h urine collection with measurement of creatinine to verify adequacy of the collection; and (3) timed (4 h or overnight) urine collections.

Considerations: South Asians are very prone to obesity and type 2 diabetes. This explains their susceptibility for central obesity and insulin resistance. It also indicates the higher rates of end-stage diabetic nephropathy in migrant South Asians[54]. They have a three times higher risk of developing diabetic nephropathy and an almost 40-fold increased risk for end-stage diabetic nephropathy when compared with Caucasians[55]. Prataap K *et al*[56] shows that central obesity is an early and independent risk factor for increased albuminuria in normoglycemic South Asian subjects.

Recently, Michiaki Fukui *et al*[57] showed the association of serum bilirubin level with microalbuminuria and subclinical atherosclerosis in patients with type 2 diabetes. Serum bilirubin concentrations were significantly lower in patients with cardiovascular disease (CVD). They were an independent determinant of CVD and had a significant inverse correlation to the log urinary albumin excretion.

Table 2 Expected results for microalbuminuria

Tests	Normal	Microalbuminuria	Macroalbuminuria
24 hr protein (mg)	< 150	< 500	⩾ 500
24 hr albumin (mg)	< 30	30-300	> 300
Timed collection for albumin (μg/min)	< 20	20-200	> 200
Spot sample collection for albumin (μg albumin/mg creatinine)	< 30	30-300	> 300

PROSPECTIVE FUTURE MARKERS

Several biochemical markers have the potential to be markers of CKD progression. These parameters might reflect diminished glomerular filtration, disturbances in tubular function or unknown contributors to kidney function that are unrelated to glomerular or tubular function. The evidence is still too sparse for most of these markers to be recommended for broad clinical use in the diagnosis of CKD progression. The emerging parameters associated with CKD progression must be studied further to determine whether they are causally related to progression of CKD or whether they simply predict the probability of progression. Few of these are as follows.

Retinol-binding protein 4

Retinol-binding protein 4 (RBP4) is a small visceral protein (approximately 21 kDa), mainly synthesized in the liver and catabolized in the kidneys after glomerular filtration[58]. RBP4 is complexed by transthyretin before delivering its ligand retinol to the target tissues[59]. RBP 4 was initially reported as an adipokine that impairs insulin sensitivity. The concentrations of this adipokine were found to increased in human subjects with impaired glucose tolerance (IGT) and type 2 diabetes compared with normal glucose tolerance subjects[60].

In prior studies, urinary RBP 4 excretion is found to be increased in early diabetic nephropathy and might even be a marker of early renal damage preceding microalbuminuria[61]. Andrea Henze *et al*[62] evaluated the influence of eGFR on RBP4 level and found that gradual elevation of RBP4 serum levels was accompanied by decline in eGFR in both, type 2 diabetic and non-diabetic subjects. No influence of type 2 diabetes mellitus or other parameters of diabetes (HbA1c, fasting serum glucose, BMI) on RBP4 serum concentration was seen. The association of RBP4 with several other metabolic parameters has been studied but limited studies are available on the relationship between this adipokine and mild to moderate decrease in GFR.

Adiponectin

Adiponectin is a recently discovered 30 kDa protein exclu sively secreted by adipocytes and is present at concen trations of 5-30 μg/mL in healthy humans. It is consid ered to be an important modulator of insulin sensitivity, dyslipidemia with anti-inflammatory properties[63,64].

Julie Lin *et al*[65] found the inverse correlation of serum adiponectin with the presence of renal dysfunction in men with type 2 diabetes, the majority of whom had well-preserved eGFR (87% had eGFR > 60 mL/min per 1.73 m^2).Adiponectin was 2.5 times higher in hemodialysis patients (15.0 *vs* 6.3 μg/mL, $P < 0.0001$)[66] and three times higher in pediatric peritoneal dialysis patients when compared with healthy control subjects.

Connective tissue growth factor

Connective tissue growth factor (CTGF) is a 36 to 38 kDa polypeptide with functions in extracellular matrix production and other profibrotic activity mediated by transforming growth factor-β1. Other biological functions of CTGF include angiogenesis, chondrogenesis, osteogenesis, and cell adhesion, migration, proliferation, and differentiation[67]. The upregulation of CTGF has been observed in human and experimental diabetic nephropathy[68].

Nguyen *et al*[69] revealed that urinary CTGF excretion is associated with urinary albumin excretion and associated inversely with glomerular filtration rate, both important clinical markers for severity of renal disease. Further, they have shown that[70] the plasma CTGF level correlates with rate of decline in GFR and that it is an independent predictor of both ESRD and mortality in patients with type 1 diabetic nephropathy. Baseline plasma CTGF was higher in patients with diabetic nephropathy than in patients with normoalbuminuria.

α1-microglobulin

α1-microglobulin is a 26 000-31 000 Da glycoprotein which exists in blood as a free form and or complexed with IgA and albumin. Because of its low molecular weight, the free form is filtered freely through the renal glomerular basement membrane and reabsorbed by the proximal tubular cells[71]. Hence, any proximal tubular cell dysfunction results in increased quantities of α1-microglobulin in the urine.

Urinary α1- microglobulin levels were found to be elevated in both type 1 and type 2 diabetic subjects. In type 2 diabetic subjects, α1-microglobulin excretion was directly correlated with albuminuria and HbA1c levels, and was decreased with improved glycemic control in causacians[72,73]. Similar findings have been shown in Asian population[74].

Transforming growth factor-β

Transforming growth factor (TGF)-β1 has a central role in fibrotic kidney disease and interstitial fibrosis (5). In type 2 diabetic patients, urinary TGF-β1 is elevated[75] and associated with histologically-proven severe mesangial expansion[76]. Although urinary TGF-β1 measurement has been suggested as a marker for diabetic nephropathy, not all studies have shown the association of urinary TGF-β1 with diabetic nephropathy. Eija *et al*[77] did not

find a difference in urinary TGF-β1 excretion between microalbuminuric and normoalbuminuric patients and only weak correlation was found with urinary glucose and β1-microglobulin. The association between urinary TGF-β1 and diabetic nephropathy may not be a direct one.

Liver-type fatty acid binding protein (L-FABP)

This protein is expressed in proximal tubular cells[78]. It has been shown that urinary L-FABP (U-LFABP) excretion is strongly associated with structural and functional tubular kidney damage in diabetic nephropathy[79]. Suzuki *et al*[80] performed a cross-sectional study in 356 adult type 2 diabetic patients and found a significant association between the stage of diabetic nephropathy and U-LFABP, although no significant difference between the normoalbuminuric and microalbuminuric groups was seen. Stine *et al*[81] have shown that U-LFABP, is elevated in type 1 diabetic patients compared with nondiabetic healthy control subjects and that the level further increases with micro and macroalbuminuria, reflecting increased tubular damage. There were no significant correlations between U-LFABP and sex, age, or A1C. Large studies with long-term follow-up are required to confirm these findings.

CONCLUSION

Diabetic nephropathy, especially related to type 2 diabetes, has become the single most important cause of ESRD worldwide. Management of traditional risk factors such as hypertension, hyperlipidemia, and smoking to improve cardiovascular and renal outcomes continues to be important in patients with chronic kidney disease. There is, however, growing recognition that nontraditional risk factors such as increased urinary albumin excretion, hypoalbuminemia, elevated serum creatinine levels, and/or decreased haemoglobin levels may also be important in individuals with chronic kidney disease. The RENAAL risk score for ESRD emphasizes the importance of the identification of levels of albuminuria and hypoalbuminemia as well as increased serum creatinine, and decreased haemoglobin levels to predict the development of ESRD in patients with type 2 diabetes and nephropathy. Albuminuria is a known strong predictor for ESRD, but the contribution of serum albumin, serum creatinine, and hemoglobin level further enhances the prediction of ESRD[82].

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