International Journal of Pharmacy and Biological Sciences-IJPBS™ (2020) 10 (2): 141-151
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Review Article | Pharmaceutical Sciences | Open Access | MCI Approved

Review on Diabetic Nephropathy and the Biomarkers for Its Early Detection

K G Praseeda*, V Gomathi and S Swathimol
***Assistant Professor, College of Pharmaceutical Sciences, Government Medical College Kottayam - 686008.**
V Gomathi, Professor, Vinayaka Missions College of Pharmacy, Salem.
S Swathimol, College of Pharmaceutical Sciences, Government Medical College Kottayam.

Received: 12 Jan 2020 / Accepted: 22 March 2020 / Published online: 01 April 2020
***Corresponding Author Email:** praseedakg@gmail.com

Abstract
The prevalence of diabetic nephropathy associated with end stage renal failure is increasing nowadays. Diabetic nephropathy is associated with many other complications such as cardio vascular morbidity and is one of the major reasons of mortality worldwide. Biomarkers are one of the most specific and sensitive tools for the early detection of diabetic nephropathy. Biomarker analysis is an advanced screening technology for experts. Justified and appropriate treatment can be given by biomarker analysis in clinical trials, which helps in well characterized, repeated and correct prediction of diseases. This review enumerates the significant candidate biomarkers for the early detection of diabetic nephropathy, also evidentially heightened the interest in identifying an ideal biomarker for diabetes induced kidney failure.

Keywords
Biomarkers, diabetic nephropathy, glomerulonecrosis, Nomo albuminuria.

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INTRODUCTION
Diabetic mellitus is a significant endocrine and metabolic disorder that has serious impact on human health. DM is associated with serious complications which include Diabetic retinopathy, Diabetic cardiovascular diseases and Diabetic nephropathy. Of these diabetic nephropathy (DN) or Diabetic kidney disease (DKD) is the most common and serious complication of Diabetic mellitus. Diabetic nephropathy is a condition begins with albuminuria and declining renal function in a patient with known diabetes in the absence of urinary tract infection or any other renal disease. [1] Diabetic nephropathy is the leading cause of reduced lifespan in diabetic patient. Around one third of diabetic patients will develop diabetic kidney disease in their lifetime. DN ultimately leads to End stage renal disease(ESRD) which is its most severe manifestation and if left untreated can cause death. DN is asymptomatic in early stages and Sustained microalbuminuria is the earliest warning symptom. Hypertension and some extent of dependent oedema eventually develop in most untreated patients. [2]
In later stages, patients may develop symptoms and signs of uremia (eg, nausea, vomiting, anorexia) earlier (i.e., with higher GFR) than patients without DN, possibly because the combination of end-organ damage due to diabetes (eg, neuropathy) and renal failure worsens symptoms [3].

Diabetic nephropathy is characterized by:

- Weight loss
- Poor appetite
- Worsening BP control
- Dry, itchy skin
- Needing to pass urine more often
- Fatigue
- Shortness of breath
- Swollen ankles and feet
- Muscle cramps
- Tiredness
- Difficulty in concentrating

These symptoms only appear when significantly affects kidney and cause damage. Intensive regulation of glycaemic levels and blood pressure control and use of angiotensin converting enzyme (ACE) inhibitors and/or angiotensin II receptor blockers delay, but do not prevent, the onset and progression of DN. There are several important kidney damage and disease biomarkers which helps in early detection of DKD. [4] Biomarkers are defined as characteristic factors that can be objectively measured and evaluated as an indicator of normal biologic or pathogenic processes of pharmacological responses to a therapeutic intervention. Examples of biomarkers are proteins, lipids, microRNAs, genomic, metabolic or proteomic patterns, imaging determinations, electrical signals, and cells present on a urine analysis. [5]

The earliest and most commonly used clinical index of DN is Microalbuminuria, a condition in which urinary albumin excretion rate (UAE) 30-300mg/day. However, its sensitivity and specificity for early detection of disease are limited. Microalbuminuria can revert to normoalbuminuric and the concept of non-albuminuric DN is as well documented. Therefore, it is essential to identify biomarkers with potential for earlier diagnosis and risk stratification in DN. An ideal blood or urinary biomarker should satisfy the following characteristics:

- Should be stable in the blood or urine for time consistent with routine clinical use
- Should be easy to measure with validated, reproducible technologies
- Should not interfere with other substance present in biological fluid
- Should be unaffected by chemical composition of the fluid
- Should reflect risk, injury and outcome with high sensitivity and specificity
- Should identify the specific site of injury (e.g; kidney tubule segment, glomerulus, endothelium or interstitium) [6]

ETIOLOGY AND PATHOPHYSIOLOGY OF DN

Diabetic nephropathy associated with glomerular sclerosis and fibrosis which is caused by the metabolic and haemodynamic changes. Poorly controlled diabetes can cause damage to blood vessel clusters in kidney that filters the blood. This can lead to kidney damage and causes high blood pressure. High BP can cause further kidney damage by increasing the pressure in the delicate filtering system of kidneys. High blood glucose levels can cause scarring of the glomerulus (glomerulosclerosis), as the scarring gets worse the kidneys being able to filter waste products from blood. When enough glomeruli have been damaged, kidney failure results. Hypertension, along with increases in intraglomerular capillary pressure and the metabolic abnormalities (like hyperglycaemia) also act as a cause to accelerate renal injury. [7]

Three major histologic changes occur in the glomeruli of persons with DN are,

1. Mesangial expansion which directly induced by hyperglycaemia perhaps through increased matrix production or glycation of matrix protein. It is detectable only 5-7 years after DM diagnosis.
2. Thickening of glomerular basement membrane can also occur, which is apparent within 1.5-2 years of DM diagnosis.
3. Glomerular sclerosis caused by intraglomerular hypertension (induced by dilatation of afferent renal artery or from ischemic injury induced by narrowing of the vessels supplying the glomeruli)

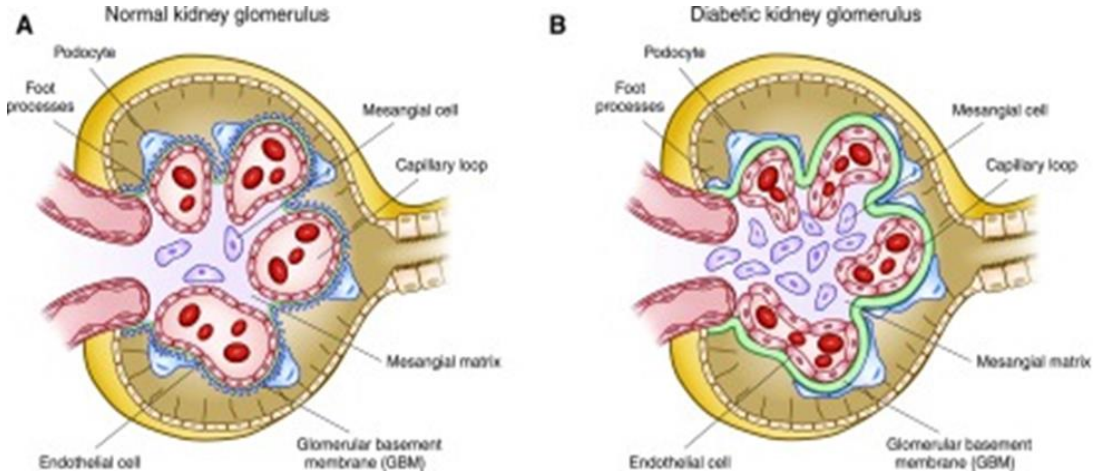

Figure.1: Schematic representation of normal and nephropathic glomerulus.

Pathogenesis commonly begins with small vessel diseases. Pathophysiology is much more complicated, involving glycosylation of proteins, hormonally influenced cytokine release (eg, transforming growth factor-beta), deposition of mesangial matrix, and alteration of glomerular hemodynamics. Hyper filtration which is an early functional abnormality, being only a relative predictor for the development of renal failure.

Hyper glycaemia causes glycosylation of glomerular proteins, which is responsible for mesangial cell proliferation and matrix expansion and vascular endothelial damage. The glomerular basement membrane basically becomes thickened. [8]

Lesions of diffuse or nodular interpapillary glomerulosclerosis are distinctive; areas of nodular glomerulosclerosis is referred to as Himmelstein-Wilson lesions. There is marked hyalinosis of afferent and efferent arterioles as well as arteriosclerosis; interstitial fibrosis and tubular atrophy also may be present. Only mesangial matrix expansion appears to correlate and progress to end-stage renal disease.

DN begins as glomerular hyper filtration (increased GFR); GFR normalizes with early renal injury and mild hypertension, which worsens over time if left untreated. Microalbuminuria, a condition of urinary excretion of albumin in a range of 30 to 300 mg albumin/day, can occur. Urinary albumin in these concentration range is called microalbuminuria because detection of proteinuria by dipstick on routine urinalysis usually requires >300 mg albumin/day. Microalbuminuria progresses to macro albuminuria (proteinuria > 300 mg/day at a variable course), over years. Nephrotic syndrome (proteinuria ≥ 3 g/day) precedes to end-stage renal disease, on average, by about 3 to 5 yrs., but this duration is also highly variable.

Other urinary tract abnormalities commonly occurring with DN that may accelerate the decline of renal function include papillary necrosis, type IV renal tubular acidosis, and UTIs. [9]

RISK FACTORS OF DN

- High blood sugar (hyperglycaemia).
- High blood pressure (hypertension).
- Smoking (nicotinism).
- Hypercholesterolemia.
- Genetic factors, family history of diabetes and kidney disease. [10]

COMPLICATIONS

- Fluid retention, which may lead to swelling of arms and legs, high blood pressure, or fluid retention in lungs (pulmonary oedema).
- A rise in potassium levels in blood (hyperkalaemia).
- Heart and blood vessel disease (cardiovascular disease), possibly leading to stroke.
- Damage of the blood vessels of the retina (diabetic retinopathy).
- Anaemia.
- Foot sores, erectile dysfunction, diarrhea and other problems related to damaged nerves and blood vessels.
- Pregnancy complications that carry risks for the mother and the developing foetus.
- Irreversible damage to the kidneys (end-stage kidney disease), eventually needing either dialysis or a kidney transplantation for survival. [11]

The pathogenesis of diabetic nephropathy is likely to be as a result of metabolic and hemodynamic abnormalities, as seen in diabetes, interacting with each other and with various reactive oxygen species-dependent pathways. Both gene regulation and activation of transcription factors are influenced by the interactions between metabolic stimuli, hemodynamic factors and reactive oxygen species generation in diabetes. The consequences of this molecular activation or inhibition are functional and

structural changes leading to the classical hallmarks of diabetic nephropathy. [12]

DIAGNOSIS

The diagnosis is done for patients with suspected diabetes who have proteinuria, particularly if they have diabetic retinopathy (indicating small vessel disease) or risk factors for DN. Other renal disorders should be considered if there are any of the following symptoms: [13]

- Heavy proteinuria with only a brief history of diabetes
- Absence of diabetic retinopathy
- Rapid onset of heavy proteinuria
- Gross haematuria
- RBC casts
- Rapid decline in GFR
- Small kidney size

Biomarkers are like the roadmap and markers in a lifelong journey of health and sickness until death. The power of biomarkers is in their predictive value, since you can start to make improvements before you actually get sick. Biomarkers play a major role in the early detection of DN.

Biomarkers of all types have been used by generations of epidemiologists, physicians, and scientists to study human disease.

Advantages of biomarkers

- ✓ Objective assessment
- ✓ Precision of measurement
- ✓ Reliable; validity can be established
- ✓ Less biased than questionnaires
- ✓ Disease mechanism often studied
- ✓ Homogeneity of risk or disease

Disadvantages

- ✓ Timing is critical
- ✓ Expensive (cost for analyses)
- ✓ Storage (longevity of samples)
- ✓ Laboratory errors
- ✓ Normal range difficult to establish
- ✓ Ethical responsibility. [14,15]

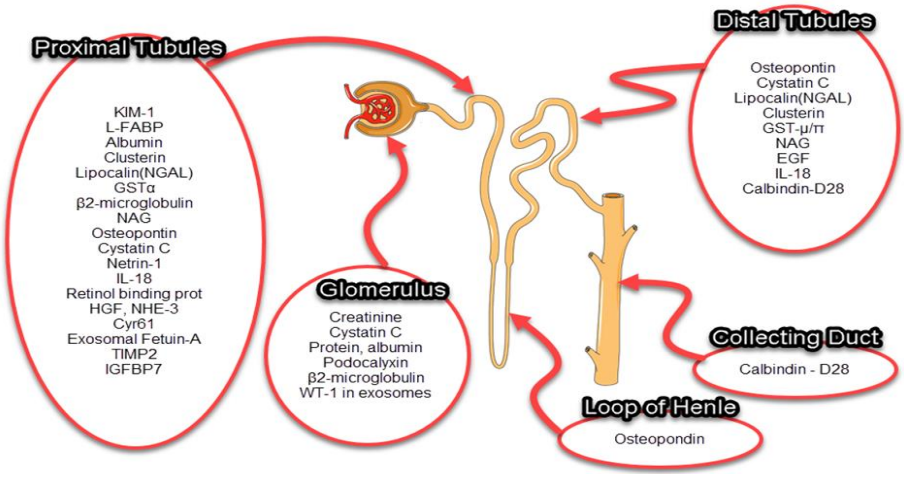

Figure.2: Candidate biomarkers for diabetic nephropathy. [16]

❖ BIOMARKERS FOR GLOMERULAR DYSFUNCTION

Glomerular filtration rate (GFR) is the best marker of renal excretory function. The current gold standard methods for determining GFR in the research setting are insulin and 51Cr-EDTA plasma clearance. The time-consuming and labour intensive nature of these techniques, as well as requirement of experienced personnel, however, mean that they are not routinely available in clinical practice. Here the most commonly used index for assessment of GFR is serum creatinine, although its sensitivity is poor in the early stages of renal impairment, as by the time an increase in serum level is detectable, a significant decline in GFR has already taken place. Cystatin C (CysC) based assays in estimating GFR for clinical trials in DN offer an alternative approach due to the complexity and time-consuming nature of other reference test methods. [17] This 13.3 kDa plasma protein is freely filtered through the glomerulus and reabsorbed and catabolised by tubular cells to such a degree that it does not return to the blood in an intact form. Numerous studies have validated CysC as a marker of renal function. Its levels are well correlated with GFR and unlike serum creatinine, are unaffected by muscle mass. In addition, CysC levels not only correlate with progression of nephropathy, but also show a more sensitive marker of early DN. [18]

(1) Cystatin C

Cystatin C, a cysteine protease inhibitor, is freely filtered by the renal glomeruli, metabolized by the proximal tubule and identified as a promising marker of renal failure. The cystatin C levels of serum and urine could be useful markers for renal dysfunction

in type 2 diabetic patients with norm albuminuria. It was more sensitive and accurate for the estimation of glomerular filtration rate (GFR) than other markers. Serum cystatin C is the most valid marker to estimate the GFR, rather than serum creatinine, and to predict progression of renal dysfunction. An increase in urinary cystatin C, independent of serum cystatin C, finds to be suggestive of renal tubular damage. In our review, urinary cystatin C is a predictor of renal impairment independent of serum cystatin C. Thus, urinary cystatin C also plays some role in predicting renal decline independent of serum cystatin C, although serum cystatin C itself, an indicator for the estimation of GFR, and is very important for predicting renal decline. [19]

(2) Transferrin

Transferrin, which is a plasma protein with a slightly greater molecular weight (76.5 kDa) than albumin. Elevated urinary transferrin excretion has been observed in patients with diabetes compared with healthy controls, even in in the absence of albuminuria. Transferrinuria has been shown to correlate with UAE and seems to increase in parallel with it. Reduced glomerular polyanion in diabetic nephropathy would be likely favours an increased transferrin excretion because the electrostatic repulsion is diminished. [20] Recently, sensitive methods to detect minute amounts of urinary transferrin have been developed, and elevated urinary transferrin excretion has been reported in both IDDM and NIDDM. Urinary transferrin was significantly increased in DM2 patients with MA. It was independent of diabetes time duration and glycemic control. According to the results, the level of urinary transferrin excretion can be used as an early biomarker of diabetic nephropathy. [21]

(3) Type IV collagen

Type IV collagen is a normal constituent of mesangial matrix as well as tubular and glomerular basement membranes, having a molecular weight of 540 kDa. Both serum and urine levels have been shown to be elevated in patients with diabetes. Urinary type IV collagen excretion has been shown to correlate closely with degree of UAE, as well as diabetes duration, blood pressure and serum creatinine. Significantly higher excretion of type IV collagen has been found even in normoalbuminuric diabetic patients as well as patients with impaired glucose tolerance, suggesting that this may serve as an early indicator of DN, preceding the onset of MA. Type IV collagen may also play a role in differentiating DN from other non-diabetic kidney diseases, as the ratio of type IV collagen to albumin has been found to be significantly higher in DN in comparison to other glomerulopathies.[22]

(4) Ceruloplasmin

Ceruloplasmin is a 132 kDa acute phase protein with well characterized functions in the metabolism of copper and iron. It has been suggested that ceruloplasmin may leak through glomerular capillary walls in DN and evidence confirms increased excretion in both impaired glucose tolerance and diabetes compared with healthy controls. Increased urinary ceruloplasmin excretion has also been demonstrated in normoalbuminuric patients with diabetes. [23]

(5) Fibronectin

Fibronectin is a high molecular weight (440 kDa) Plasma glycoprotein which is mainly produced by endothelial cells and fibroblasts which plays a role in cell adhesion to vascular endothelium. Fibronectin biosynthesis is increased in patients with diabetes and studies have suggested that plasma levels correlate with retinopathy and MA. Increased urinary levels of fibronectin have also been found in type 2 diabetic patients in comparison with healthy controls, as well as in subjects with MA compared to normoalbuminuric subjects. However, there is only a weak positive correlation between plasma fibronectin and urinary albumin levels perhaps limiting its potential usefulness as an early marker of DN, and there is no published evidence comparing urinary fibronectin with UAE in terms of predictive value for diabetic nephropathy. [24]

❖ BIOMARKERS OF OXIDATIVE STRESS

Hyper glycaemia encourages mitochondrial electron transport chain which leads to the generation of excessive reactive oxygen species (ROS). It happens via the formation of the advanced glycation end products (AGEs) and activation of the polyol pathway, hexosamine pathway, protein kinase C (PKC) and angiotensin II. Then, oxidative stress is getting initiated or enhanced by ROS, and later it causes the inflammatory response and formation of fibrosis. Further, intracellular signaling pathway activation, lipid metabolism abnormality, increased growth factors and pro-inflammatory cytokines, renin-angiotensin-aldosterone system (RAAS) activation, insulin signaling impairment, and systemic and glomerular hypertension also contributes to the occurrence and progression of DN. [25] AGE is directly affected to the development of diabetes complications. It's intuitive that the presence of AGE-modified protein fragments in urine may also herald early tubular dysfunction, as AGE-modified proteins generally undergo glomerular filtration and subsequent catabolism at the proximal tubule. [26]

(1) 8-oxo-7,8-dihydro-2'-deoxyguanosine(8-OHdG)

Oxidative DNA damage will produce 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-OHdG), mainly after specific enzymatic cleavage after ROS-induced 8-hydroxylation of the guanine base in nuclear and mitochondrial DNA. Its urinary concentration serves as an index of oxidative stress, as it will be excreted into urine without being further metabolized. Urinary excretion appears to correlate closely with the severity of DN and retinopathy, as increased concentrations of 8-OHdG have been described in both urine and mononuclear cells of diabetic patients. [27]

(2) Pentosidine

The major molecular structural components of AGEs which acts as a marker of their formation and accumulation is pentosidine. When we compare patients with diabetes to those with healthy controls, urinary excretion of Pentosidine has been shown to be higher in patients. Patients with DN have been demonstrated with increased urinary and plasma Pentosidine levels. In addition to DN, associations between serum Pentosidine levels and diabetic retinopathy, hypertension and hyperlipidaemia, found possible to be shown as a marker of microvascular complications of diabetes. Studies between diabetes patients with macro albuminuria compared to those with controls found an increased median urinary Pentosidine excretion in patients. [28]

❖ **BIOMARKERS OF INFLAMMATION**

Individuals will display features of low-grade inflammation for years before clinically detectable disease, even they progress to DN. Then, the following will be as potential markers of DN, cytokines and other components involved in the process of inflammation and endothelial damage. [29]

(1) Tumour necrosis factor(TNF-α) and Interleukin (IL-6)

TNF-α mediates its effects *via* two distinct receptors, TNF receptor 1 (TNFR1) and TNFR2, which are both membrane bound and also can be found in serum in soluble form. Serum levels of both these receptors have been shown to correlate with GFR in diabetic patients independently of albuminuria status. More recent data suggest that serum concentrations of TNFR1 and TNFR2 have potential as predictors of progressive renal disease in diabetes. Patients with TNFR levels in the highest quartile show TNF-α and IL-6 are two major pro-inflammatory cytokines that stimulate the acute phase response by triggering production of other proteins such as CRP and AGA. [30] Patients with DN have higher serum and urinary concentrations of TNF-α than healthy controls or normoalbuminuric subjects. Urinary TNF-α excretion also appears to be increased in diabetes patients with micro- or macro albuminuria compared to normoalbuminuric patients, with one study reporting an increase of 90% between normo- and microalbuminuric patients. Significantly elevated cumulative incidence of reaching stage 3-5CKD over 12 years of follow up compared with those in the lower quartiles. This has been shown in both type1 and type 2 diabetes, in the presence or absence of proteinuria. Serum IL-6 has been shown to be elevated in patients with diabetes compared to control subjects, as well as between normo-, macroalbuminuric and overtly proteinuric patient groups. In addition, IL-6 has been linked to glomerular basement membrane thickening. Furthermore, association has been demonstrated between circulating levels of both TNF-α and IL-6 and micro- and macro vascular complications of diabetes. [31]

(2) Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF) is a potent cytokine that induces angiogenesis and increases endothelial permeability. It adversely affects the glomerular filtration barrier by enhancing its permeability to macromolecules and exacerbating proteinuria. Urinary VEGF excretion appears to be elevated in patients with diabetes, even at the normoalbuminuric stage. Work in type 2 diabetes demonstrated that urinary VEGF concentration increases with DN stage. [32]

❖ **BIOMARKERS OF PODOCYTES INJURY**

Podocytes (or visceral epithelial cells) are highly specialized cells of neuroepithelial origin that wrap around the capillaries of the glomerulus. The long processes of the podocytes wrap around the capillaries and between these processes are small slits in which contain a slit diaphragm. An increasing number of proteins have been identified to be present in these foot projections that wrap around the capillaries. [33] Nephrin is a zipper-like protein that plays a functional role in the structure of the slit diaphragm. The spaces between the teeth of the zipper allow, in a selective manner, small molecules, such as glucose and water, to traverse, but are too small to allow large proteins to cross. Indeed, defects in nephrin have been reported to be responsible for the massive albuminuria that occurs in the Finnish type of congenital nephritic syndrome and kidney failure. Studies have shown that a decline in the number of podocytes and disappearance of foot processes often occur in the early stages of DN due to apoptosis or shedding of podocytes. Therefore, urinary podocytes and their specific protein products may be regarded as potential biomarkers of podocyte injury. Currently, the studies focused on the podocyte-specific protein products because it

was difficult to detect urinary podocytes directly. [34] Studies showed that urinary mRNA levels of podocin, synaptopodin and nephrin in DN patients were extremely higher than those found in control subjects by real-time quantitative PCR. These results were also proved by renal biopsy. Also, synaptopodin level was positively correlated with urinary albumin excretion and serum creatinine concentration while negatively correlated with GFR. Researches revealed that urinary synaptopodin level of type 2 diabetes mellitus patients complicated by nephropathy was higher when compared to control subjects, even before the occurrence of proteinuria and associated with the level of urinary albumin and HbA_{1c}, indicating that synaptopodin was a biomarker with high sensitivity to podocyte injury in diabetic patients. [35]

❖ BIOMARKERS OF TUBULAR DYSFUNCTION

(1) Neutrophil gelatinase-associated lipocalin (NGAL)

Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker of renal tubular injury, upregulated in distal tubules and collecting duct, is extensively evaluated for acute kidney injury (AKI). It is a 25 kDa glycoprotein with 178 amino acid belonging to lipocalin superfamily. It is a constituent of specific granules and exists in neutrophil as a part of NGAL-gelatinase complex. It is involved in antimicrobial defence mechanism and upregulated in systemic bacterial infection. It plays a protective role in epithelial injury by its anti-apoptosis effect. As it is not produced by burnt out nephron, it is supposed to be a marker of active injury and represents mass of salvageable nephrons. From studies, there was significant difference in median value of sNGAL and uNGAL between diabetes patients compared to well-matched control, suggesting that NGAL can be a useful marker of onset of DN even when serum creatinine is normal range. [36] This may be due to protective response of NGAL in response to metabolic and hemodynamic stress. Intergroup comparison among three groups of T2DM patients shows that difference was significant with highest median value in macro albuminuria and lowest in norm albuminuria, which suggest that sNGAL and uNGAL can be used to predict progression of DN. Again, sNGAL and uNGAL correlates positively with albuminuria suggesting that former correlates with severity of renal involvement. This concludes that NGAL can be used as a marker to stratify diabetes nephropathy into different stages. Tubular injury may precede glomerular injury in diabetic patients, and NGAL can be used as biomarker to diagnosed DN even earlier to incipient nephropathy. Both sNGAL and uNGAL can predict albuminuria and be used as a noninvasive tool for diagnosis, staging, and progression of DN. [37]

(2) Kidney Injury Molecule-1(KIM-1)

Kidney injury molecule-1 (KIM-1) is a type 1 cell membrane glycoprotein, which contains, in its extracellular portion, immunoglobulin- and mucin-like domains, with *N*- and *O*-glycosylation sites. KIM-1 expression is undetectable in normal kidneys but the mRNA and protein are markedly upregulated with acute kidney injury. [38]

There are many reasons to consider that KIM-1 may be released into the circulation after kidney proximal tubule injury. With injury, tubular cell polarity is lost, such that KIM-1 may be released directly into the interstitium. Further, increased trans epithelial permeability after tubular injury leads to back leak of tubular contents into the circulation. Also, altered microvascular permeability is an important contributor to the pathophysiology of kidney injury. The actin cytoskeleton architecture is disrupted in renal microvascular endothelial cells, with loss of cell-cell and cell-matrix adhesion junctions, and endothelial cells are detached from the basement membrane; this facilitates KIM-1 movement into the circulation. Increased levels of KIM-1 can be detected in the blood and serve as a biomarker of kidney injury. [39]

(3) N-acetyl-beta-glucosaminidase(NAG)

N-acetyl-b-d-glucosaminidase (NAG) is a lysosomal enzyme which is predominantly located in the renal tubules. It cannot be filtered from blood through an intact glomerular membrane due to its high molecular weight (140 kDa), thus its activity detected in urine reflects tubular dysfunction. [40] Urinary NAG activity is increased in a variety of tubulointerstitial diseases. It is elevated in populations with diabetes compared to controls, even in normoalbuminuric patients. It correlates with the degree of UAE and excretion of transferrin and creatinine. Finally, significant increases in NAG excretion have been reported in type 2 diabetic patients with both micro- and macro vascular complications and in fact NAG levels have been attributed comparable diagnostic value to UAE in this regard. [41]

Studies showed that urinary NAG had higher sensitivity than SCr and met the criteria for detecting glomerular and tubular dysfunction as screening tests for early diagnosis of DN, demonstrating usefulness of urinary NAG as a biomarker for early DN in diabetes. Another study from type 2 diabetic patients found that urinary NAG was significantly higher in all patient groups than in controls and in microalbuminuric than in normoalbuminuric patients. Thus urinary NAG excretion might be a useful biomarker of early renal injury in diabetic

patients, even at the normoalbuminuric stage. A more recent study found that urinary NAG was significantly higher in type 2 diabetic patients with normo-, micro- and macro albuminuria than in non-diabetic controls, and its value increased in parallel with the severity of renal involvement. Significant positive correlation was observed between urinary NAG, SCr and HbA1c, suggesting that urinary NAG could be used as a useful biomarker reflecting the degree of renal impairment in DN. [42]

(4) Copeptin

Arginine vasopressin (AVP) is one of the main hormones of the hypothalamic–pituitary–adrenal axis, and it is mainly stimulated by hyperosmolarity. AVP plays deleterious renal effects, inducing hypertension, glomerular hyper filtration, albuminuria, and glomerulosclerosis, whereas its inhibition, by drinking water or by V2 antagonism, led to a Reno protection. The direct measurement of AVP in humans is problematic, due to its bond with platelets and its instability in isolated plasma. Copeptin, the C-terminal portion of the AVP precursor and released into the circulation from the posterior pituitary gland in equimolar amounts with AVP, represents a measurable substitute. It is easier to measure copeptin rather than AVP in serum due to its superior stability. [43] Previous studies have demonstrated high functional correlation between copeptin and mature AVP supporting it as an appropriate surrogate marker in lieu of AVP. Copeptin is partly cleared by the kidneys and hence, the level of copeptin is higher in chronic kidney disease (CKD) or end-stage renal disease (ESRD) patients, compared to those with preserved kidney function. Serum copeptin not only predicts the outcome of renal adverse events, but may serve as a sensitive indicator of early decline in renal function. [44]

❖ BIOMARKERS OF MESANGIAL EXPANSOIN AND FIBROSIS

Progressive renal fibrosis, can also occur in individuals, eventually reducing functioning renal mass. Fibrosis arises, in part, through 'activation' of renal fibroblasts to secrete and remodel the extracellular matrix. In nondiabetic kidney disease, studies strongly suggest that matrix-producing effector cells also arise from phenotypic transitions in mesangial cells and tubular epithelial cells. Profibrotic switches in the phenotype of mesangial and epithelial cells might also promote fibrosis in diabetic nephropathy. [45]

Fibrosis is one of the pathological features of diabetic complications caused by extracellular matrix alterations and mesangial expansion. Hyper glycaemia up-regulates the expression of transforming growth factor-β1 (TGF-β1), which is considered to be the most crucial cytokine in glomerulosclerosis and tubulointerstitial fibrosis. Studies showed that serum TGF-β1 level of patients with microalbuminuria was significantly higher than that of patients with normoalbuminuria and control subjects. Interestingly, urinary levels of TGF-β1 are already elevated in normoalbuminuria subjects and gradually increase along with DN progression, so that TGF-β1 was considered a sensitive biomarker in the early phase of diabetic nephropathy. [46]

❖ BIOMARKERS OF RAAS ACTIVATION

(1) Angiotensinogen

The regulation of blood pressure, sodium and water balance, and cardiovascular and renal homeostasis are maintained by the renin- angiotensin-aldosterone system in body. The excessive activation of the RAAS in diabetic nephropathy results in progressive renal damage. In patients with chronic glomerulonephritis, high degree of angiotensinogen, produced by liver was reported in a previous study. [47] The following study found that urinary angiotensinogen excretion of type 2 diabetes mellitus patients with microalbuminuria and macro albuminuria were both significantly increased compared to control subjects, as well as to normoalbuminuric patients, suggesting that angiotensinogen appeared prior to the establishment of albuminuria. Also, angiotensinogen level shows a strong association with urinary albumin excretion, which is an indicator of the severity of kidney damage in diabetic patients. Angiotensinogen may be a promising biomarker in the early stages of DN due to its high sensitivity and specificity in diagnostic analysis of diabetic nephropathy. Urinary angiotensinogen was higher in microalbuminuric and macroalbuminuric diabetes than in controls. In diabetes with normoalbuminuria, urinary angiotensinogen was also higher than in controls. [48]

(2) Immunoglobulin G/M

Immunoglobulin G or M (IgG or IgM) are large proteins synthesized and secreted by plasma cells, and their appearance in urine indicates that a large, nonselective pore exists in the glomerular capillary wall. The urinary IgG and IgM of type2 DM patients seems to be having significantly higher degree, and found to have albuminuria than the healthy control subjects. The study suggested that elevated urinary levels of IgM and IgG2 might be more sensitive markers of nephropathy than albuminuria in patients with type 2 diabetes and antihypertensive therapy. [27]

Another study found that urinary IgG excretion was increased significantly in diabetic patients compared to healthy controls and was further increased

significantly in chronic renal failure patients with respect to the clinical stage of nephropathy suggesting the significant role of urinary IgG excretion as a biomarker for early type 2 DN. The rate of progression to microalbuminuria was significantly higher in patients with increased Urinary-IgG than in patients without increased Urinary-IgG which indicated urinary IgG levels could be a useful biomarker for development of DN in normoalbuminuric type 2 diabetic patients. [32]

(3) Alpha-1-microglobulin

Alpha 1-microglobulin (α1-microglobulin) is a 27-kDa glycoprotein which is filtered freely by the glomeruli and reabsorbed by the proximal tubule. Increased urinary excretion of α1-microglobulin has been suggested as an early biomarker for screening DN. In one study, raised urinary α1-microglobulin was found in 33.6 % of patients with normoalbuminuria, 53.6% microalbuminuria and 64.5% macro albuminuria, suggesting that urinary α1-microglobulin might be useful for the early detection of DN in diabetics. Urinary α1-microglobulin was related to duration, severity and control of diabetes, and was directly related to albuminuria, indicating that it is a good biomarker of the severity of renal impairment in type 2 diabetic patients. Thus, urinary α1-microglobulin enables non-invasive assessment of DN development at an early stage, while more studies are needed to confirm its utility in type 2 DN. [43]

CONCLUSION

As the increasing prevalence of diabetes reaches epidemic proportions worldwide, diabetic nephropathy and associated end stage renal failure will be an unavoidable major health burden to the population. Diabetic nephropathy is also associated with an increased risk of cardiovascular morbidity and mortality. Therefore, it is essential to detect the onset or progression of DN as early as possible. Biomarkers indicate a problem before a disease or health problem appears. Biomarkers often reveal a biological situation that we might not have any observable or felt symptoms for. There are several biomarkers which helps in the early detection of diabetic nephropathy by being appear at the normoalbuminuric stage itself. They possess high sensitivity and specificity and thus became potential tool for the early detection of Diabetic nephropathy.

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