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URINARY BIOMARKERS FOR EARLY DETECTION OF DIABETES AND DIABETIC RENAL COMPLICATIONS

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ABSTRACT

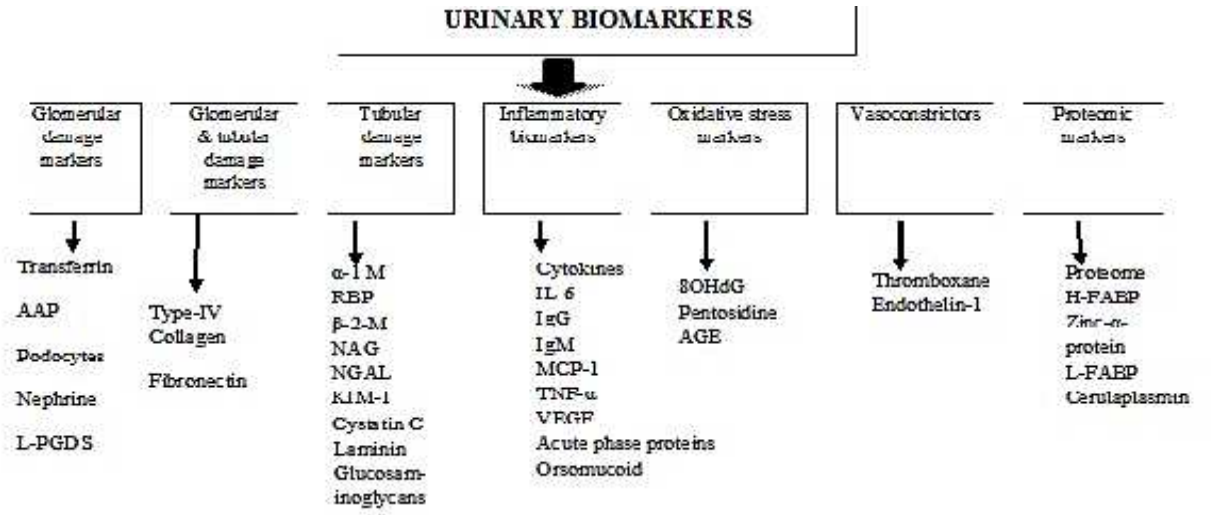
Diabetes is also associated with the development of renal complication such as chronic kidney disease, Retinopathy and Diabetic Nephropathy. Although Albuminuria is widely being used as a marker of Glomerular and Tubular damage and has attained the current gold status in diagnosis but other biomarkers have been found that indicate renal damage prior to albuminuria and also predict the progression of different stages of diabetes and progression of normoalbuminuria toward microalbuminuria and macroalbuminuria. This review gives an overview of Tubular damage markers, Glomerular damage markers, Oxidative stress markers and Proteomic markers that can be more specific and sensitive and act as early markers than Albumin.

Key words: Diabetic Nephropathy, Albuminuria, Glucose Tolerance Test, Transferrin

INTRODUCTION

Diabetes is a chronic disease characterized by blood glucose level increase or hyperglycemic condition as the pancreatic-β-cells produce insufficient insulin. It is one of the most important health issues that the modern science is facing in the 21st century. According to a survey it affects about 366million of the world's population and till 2030 the number may increase up to 522million(Federation, 2011). DM is also followed by other medical problems especially chronic kidney disease, Diabetic Nephropathy and End-stage renal failure.

These conditions may increase the complications of disease. It has also been observed that about 40% of DM patients develop DN. So, for better control and treatment of the disease early diagnosis is required. Plasma glucose is measured by three test panel i.e., "Random Plasma Glucose", "Fasting Glucose Test" and "Oral Glucose Tolerance Test"(Waugh *et al.*, 2007).Studies have shown that other marker such as Glomerular damage, tubular damage, growth factors, inflammatory mediators, oxidative stress and RAAS system molecules show higher values prior to disease and during disease progression their levels indicate different stages of the disease. This review gives a brief introduction to those markers that may serve as sensitive and more reliable markers present in the literature for diabetes and diabetic nephropathy preceding the disease.



MARKERS OF GLOMERULAR DAMAGE

Albumin

Albumin is a 65kD most abundant plasma protein that originates from liver and functions as oncotic pressure regulator, maintainer of volume of blood, transporter of molecules such as ions, drugs, hormones, bilirubin and vitamins. It is filtered freely by the renal tubules through glomerulus. Elevation in urinary level of albumin is termed as Albuminuria and it is considered as the most well-established and early marker of Glomerular damage and an independent risk factor of both renal and cardiovascular disease in diabetic population. Albumin has a direct role in the progression of kidney diseases.Albuminuria can be classified into three categories: Normoalbuminuria, defined as UAE <30mg/day or per g of creatinine. Microalbuminuria, representing UAE between the range of 30-300mg/day and Macroalbuminuria, characterized by UAE >300mg/day. All the three categories diagnose different stages of diabetes and diabetes associated with renal problems. Microalbuminuria is most important for the detection of type-2 DM as well as type-1 DM and its efficiency has been observed in population. 42% nephropathy risk increase has been observed in patients who developed microalbuminuria.

Transferrin

Transferrin is an iron binding serum protein whose molecular weight is nearly equal to albumin (Mol. Wt 76.5kD)(Kazumi *et al*., 1999). Transferrin is responsible for the transfer of ferric ions from the intestine and liver to all the proliferative cells in the body. Urinary excretion of Transferrin is regarded as a good biomarker of glomerular damage in type2 DM patients havingnormoalbuminuria in comparison to controls. It is suggested by several reports that urinary transferrin indicates a good linear relation with UEA in DM patients another study predicts abnormal urinary transferrin/creatinine ratio in 61% of normoalbuminuric, 95% of microalbuminuric as well as 100% of macroalbuminuric patients as shown in Table 1. This could make transferrin a much more sensitive marker than albumin and is good for the prediction of onset of Nephropathy(Hellemons *et al*., 2012). Studies have indicated an increased risk of microalbuminuria in patients with normaloalbuminuria if there is an elevated level of transferrin excretion similarly patients will develop microalbuminuria more frequently if the initial urinary transferrin level was high. It was also reported that 31% of type2 DM patients have developed microalbuminuria with transferrinuria while in 7% patients without transferrinuria.

Although it is useful as a early marker of DN than albumin but still it is less specific due to its increase in primary glomerulonephritis, vascular complications of type 2 DM patients (such as coronary artery disease and diabetic retinopathy) and CVD(Quiroga *et al.*, 2015).

Table 1: Patients percentage showing excretion of corresponding biomarkers

Biomarkers	Prediction	Percentage of Normo-albumunic patients	Percentage of Micro-albuminuric patients	Percentage of Macro-albuminuric patients	References
Transferrin	Glomerular damage	61%	95%	100%	(Zhou *et al.*, 1997)
Podocytes	Glomerular damage	53.8%	64.7%	66.7%	(Yamazaki *et al.*, 1995)
Nephrine	Glomerular damage	53%	71%	90%	(Kandasamy *et al*., 2014)
Alpha-1-Microglobulin	Tubular damage	33.6%	53.6%	64.5%	(Petrica *et al.*, 2010)
RBP	Tubular damage	-	90.9%	-	(Park *et al*., 2014)
NAG	Tubular damage	34%	63.7%	49.5%	(Ambade *et al.*, 2006)
AGE	Oxidative stress	50%	85%	-	(N. Turk *et al*., 2004)

Alanine aminopeptidase

High levels of Alanine aminopeptidase in urine of type 2 diabetic patients in comparison to controls. With normal albumin excretion reportedin 22.4% patients. Later on a study showed insignificant differences between controls and normoalbuminuric patients but was significant between albuminuric and normoalbuminuric or control patients(Farvid *et al.*,2007).

Podocytes

Podocytes are called as Glomerular epithelial cells and they are the key structural element of glomerular filtration barrier. Evidences showed the role of structure and function of podocytes in Diabetic nephropathy before onset of albuminuria(White *et al*., 2002) hence the podocyte injury plays a important role in the progression of DN, the appearance of podocyte lesions during DM is referred as Podocytopathy. Podocyte injury can be monitored by using Podocyte injury urinary biomarkers and podocyte-specific proteins.In microalbuminuric patients urinary podocyte is higher and is correlated to osteopontin and IgM in urine. In DM patients, Podocalyxin presented a higher level in 53.8% of Normoalbuminuric patients, 64.7% of microalbumunic patients and 66.7% of macroalbuminuric patients as sited in Table 1 and hence it might be a useful and early biomarker of podocyte injury in DM patients another study showed its correlation with urinary NAG and beta-2-microglobulin values(Shoji *et al.*, 2016).

Nephrine

Podocytes as discussed above are an important part of glomeular filtration barrier and any damage to them can lead to Podocytopathy which is also considered as Diabetic nephropathy(Weil *et al*., 2011). Higher excretory levels of podocyte associated proteins are observed in normoalbuminuric DM patients as compared to control subjects reporting renal damage in early stage of diabetes.Nephrine with180kD molecular weight is a transmembrane protein present in glomerular podocytes and its excretion in urine indicates glomerular injury as podocyte lesions and progression of DN showing it as a potential marker of glomerular injury(Kandasamy *et al.*, 2014). In type1 DM nephrinuria occurs before microalbuminuria. Nephrinuria was reported in son type2 DM patients with normoalbuminuria in correlation with ACR and eGFR(Petrica *et al*., 2014).

In a study 53% normo-, 71% micro- and 90% of macroalbuminuric diabetic patients were reported to have nephrinuriathat is mentioned in table 1 and it was related with increasing UEA

while another study found 100% patients with micro- and macroalbuminuria and 54% patients even with normoalbuminuria had nephrinuria regarding it as an early biomarker for type2 DM subjects. High sensitivity and specificity is offered by the presence of nephrine in urine and thus it is suggested for early detection of DN(do Nascimento *et al*., 2013).

Lipocalin- Type Prostaglandin-D Synthase

It is abbreviated as L-PGDS. A biomarker related to glomerular capillary wall lesions indicating their increased permeability. It is less relevant as an early biomarker of diabetes but is used to predict renal lesions(Uehara *et al.,* 2009).

MARKERS OF GLOMERULAR –TUBULAR BASEMENT MEMBRANE DAMAGE

Type IV Collagen

It is the main structural constituent of glomerular and tubular basement membranes as well as mesangial matrix. Both glomerular level and mesangial level lesions are produced in DN causing typeIV collagen excretion in urine and is an indicator of renal injury(Fiseha, 2015). Type1 DM patients showed high levels of typeIV collagen even with normoalbuminuriaserving as a marker of DN. Patients with impaired glucose tolerance also indicated high levels of type IV collagen excretion.Significantly high excretion levels of typeIV collagen have been reported in type2 DM patients with micro- as well as normoalbuminuria (lower than microalbuminuria) as compared to healthy subjects in correlation to albuminuria. Chi-square study indicated it to be more sensitive than UAE in detection of renal damage in type2 DM patients(Kotajima *et al.,* 2000).

In 25% of population of type2 DM developed microalbuminuria while others remained normoalbuminuric with a decrease in their type IV collagen(Kotajima *et al.,* 2000).Hence, it is a more specific indicator of DN than UAE and it differentiates between diabetic and non diabetic nephropathy.

Fibronectin

Fibronectin is synthesized by the liver, platelets and vascular endothelia and an important constituent of extracellular matrix. It plays role in platelet function, tissue healing, coagulation,cell shape maintenance and cell adhesion. In sporadical study, its elevated level was detected in type2 DM patients as compared to controls but the data is not sufficient(Perkins *et al*., 2007). Its excretion increase gradually with degree of albuminuria and glomerular lesions and further research is required to determine its relevance as compared to microalbuminuria.

MARKERS OF TUBULAR DAMAGE

Alpha 1 Microglobulin

α-1-microglobulin is a 27kD glycoprotein that filters freely through the glomerular capillary wall and as soon as it arrives at the proximal tubules it is reabsorbed and metabolized. Thus its excretion in urine indicates renal damage and it can act as an early biomarker of DN.As shown in Table 1, 33.6% of normoalbuminuric, 53.6% of microalbuminuric and 64.5% of macroalbuminuric patients showed a higher value of α-1-microglobulin excretion, showing that it might be useful in detection of early stage DN. It has a direct relation to UAE, diabetic duration and glycemic control in case of type2 DM patients. α-1-microglobulin has higher value in diabetic patients in comparison to control subjects in microalbuminuria than normoalbuminuria suggesting it a useful indicator of DN(Petrica *et al*., 2014).

Retinol binding protein

RBP is a low molecular (21kD) protein that is completely reabsorbed by proximal tubules after free filtration through the glomerulus. It is present in plasma bound to transthyretin forming a complexthat avoids its renal excretion and also in urine. The serum and urinary levels of RBP are

high in patients of DM(Novery, Susanah, and Rachmadi, 2015). Increased levels of RBP are observed in diabetic subjects as compared to controls in a positive correlation with duration of disease, glycemic control, Blood pressure (systolic), triglyceride and UAE(Abdallah *et al.*, 2011).Impaired proximal tubule function is marked by the high concentration of RBP in micoalbuminuric DM patients in comparison to normoalbuminuric and controls.
About 90.9% of microalbuminuric patients with renal complication presented a high amount of RBP with a specificity of 64.03% and sensitivity of 80.18% (Table 1), so RBP can be used as a significantbiomarker of renal damage in DM patients(Park *et al.*, 2014). Some studies also indicated a high excretion of RBP even before microalbuminuria and in normoalbuminuria in correlation to other urinary markers(Catalano *et al.*, 1996;).

NGAL

Neutrophil gelatinase associated lipocalin, a glycoprotein of about 25kDa present in kidney tubular cells(Al-Refai *et al.,* 2014). It has a protective role against renal damage. It indicates the presence of tubular lesions in normoalbuminuric DM patients.In type 1 DM NGAL increases prior to microalbuminuria while in type2 DM NGAL has high value in normoalbuminuric patients that increases progressively to micro- and macroalbuminuric patients(de Carvalho *et al.,* 2016). NGAL is positively correlated to proteinuria, cystatinC, serum nitrogen and creatinine but is inversely correlated to GFR.So it can be a significant marker to indicate evolution of DN in DM patients(Nielsen *et al.*, 2010).

Kidney injury molecule-1

KIM-1 a glycoprotein that is secreted by the proximal tubular cells and its high excretion value indicates impaired kidney function, lesions or injury at this level(Bonventre, 2014). High levels of KIM-1 have been observed prior to diabetes development and overt kidney disease as compared to nondiabetic subjects. Its presence indicates highly specific kidney damage and renal proximal tubule injury that helps in early diagnosis of DN in type 2 DM patients(Petrica *et al.*, 2014).A study indicated increased value of KIM-1 along with UAE and DM duration was found as an independent marker of low GFR and its level was high among both DN and CDK subjects as compared to controls(Tekce *et al.*, 2014).Microalbuminuric patients were reported to have a high urinary KIM-1 value as compared to normoalbuminuric as well as between normoalbuminuric patients and controls enabling an early diagnosis of DN(El-Ashmawy *et al.*, 2015). It was also positively related to kidney inflammation.

By measuring KIM-1 levels in urine “tubular phase” of renal damage could be predicted prior to albuminuria(Ucakturk *et al.,* 2016). In type 2 DM high level of KIM-1 can be predictive with normal or mildly increased UAE. It suggests KIM-1 is a sensitive biomarker of early DN even preceding albumin secretion(de Carvalho *et al.,* 2016).

Cystatin C

Cystatin C is a low molecular weight protein synthesized by the nucleated cells of the body which acts as a cysteine protease inhibitor. It is considered a better glomerular and tubular dysfunction as compared to serum creatinine concentration. It shows an increased excretion in diabetes and nephropathy prior to diabetes(Garg *et al.,* 2015).It increase in urine may serve as a (independent of serum cystatin) marker of early stage glomerular renal damage in type2 DM patients(Rao *et al.,*2014). Due to its excretion in pre-diabetic stages representing nephropathy and its association with reduced GFR, it is regarded as a good biomarker of DN.Microalbuminuric patients show significantly high urinary levels of Cystatin C as compared to normoalbuminuric patients(Garg *et al.,* 2015) and it increases with an increase in degree of albuminuria such as in macroalbuminuric patients. Thus Cystatin C might be a useful biomarker of early DN in type2 DM patients.

Glucosaminoglycans

Glycosaminoglycans excretion in urine is increased in case of normaloalbuminuric diabetic patients due the alteration of glomerular basementmembrane and the extracellular matrix as Glycosaminoglycans are their important component.They are also the biomarker of distal tubular dysfunction in diabetic patients. As they are also present at the level of the tubular basement membrane.

Beta 2 Microglobulin

All cells that express MHC class 1 antigen produce a low molecular weight 11.8kD protein called Beta 2- microglobuin(β2-M) that readily filters through glomerulous and completely reabsorbed and proximal tubules catabolize it so, an increase in β2-M reports tubular impaired function which may be reliable as a sensitive tubular injury marker and it is correlated with severity of tubulo-intestinal injury in biopsy-proven type 2 DN(Mise *et al.,* 2016).β2-M excretion in urine of type2 DM patients with nephropathy is higher than normal and is positively correlated to UAE while negative correlated to GFR(Aghamohammadzadeh *et al.,*2015).In children with type1 DM, increase in β2-M has a positive correlation with duration of disease and glycemic control.In type 2 DM, patients with poor metabolic control exhibit elevated level of excretion of β2-M as compared to those with good control.In microalbuminuric patients β2-M excretion is higher than normoalbuminuric and control subjects reporting tubular damage in early DN(Nikolov *et al.,* 2013). Increased β2-M excretion was found before UAE appearance indicating DN related to proximal tubule dysfunction.

In 23.5% of normoalbuminuric type 2 DM patientsβ2-M excretion was higher indicating that tubule dysfunction may be responsible for early DN independent of glomerular endothelial dysfunction and its excretion is increased progressivelyfromnormoalbuminuria to macroalbuminuria and β2-M is a sensitive marker of early DN and its progression.

NAG

N- Acetyl-Beta-glucosaminidase is a proximal tubular cell lysosomal enzyme with molecular weight of 130kDa involved in carbohydrate metabolism(Uslu *et al*., 2005). Urinary NAG is specific for tubular damage because the plasma NAG cannot be filtered through kidney. Increased in urinary NAG was seen in 90% of patients versus controls. 89% of increased UAE patients also show increase in NAG excretion. As inTable 1, 34% of normoalbuminuric, 63.7% of microalbuminuria and 49.5% of overall diabetic patients show an increase in NAG excretion suggesting it as an early and most sensitive marker of renal damage in DM subjects(Sheira *et al.*, 2015).

Laminin

Laminin excretion is increased in normoalbuminuric type 2 diabetic patients. Its excess is beingcorrelated with NAG (N-acetyl-beta-D-glucosaminidase) and α-1-microglobulin excretion as it is a component of the basement membrane of glomerulus.

INFLAMMATORY BIOMARKERS

Cytokines

Acute phase inflammatory reaction occurs in the presence of two cytokines TNF-α and IL-6. Theirelevated excretion was reported in type2 DM patients. 250% increase in urine concentration of TNF-α and 40% increase in urinary IL-6 was reported as they are also produced by the renal cells apart from other body cells but increase in urine concentration by different studies indicates renal injury.

Interleukin-6
Only an increase of 9% has been observed between the microalbuminuric and normoalbuminuric DM patients. Earlier studies have shown its association with DN progression in type2 DM but much more study is required for its use in diagnostics.

Immunoglobulin G
Plasma cells secrete an anionic plasma protein IgG with a molecular weight of 150kD and molecular radius 5.5nm that is larger than albumin. This causes difficulty to cross the glomerular barrier and its appearance in the urine indicates large, non-selective pore existence in capillary wall of glomerulus(Cohen-Bucay & Viswanathan, 2012). Urinary IgG is correlated with progression of glomerular lesions and its high excretion is reported in type2 DM patients(75%) with nephropathy as compared to healthy subjects. A study showed that its increase elimination predicts microalbuminuria while its excretion in normoalbuminuric patient reports development of microalbuminuria. Thus it may be helpful in early detection of type2 DN.

Studies have shown that IgG is secreted before UAE and can predict development of microalbuminuria in normoalbuminuric type2 DM patients as 47.1% of Microalbuminuric patients show with high IgG excretion levels as compared to 9% of patients without increase in IgG. So IgG can be considered as a marker of development of nephropathy in type2 DM patients(Hellemons *et al.*, 2012).

Immunoglobulin M
Plasma cells secrete IgM that is an antibody in human with largest molecular radius. Its excretion in urine indicatesimpaired kidney functioning reporting non-selective pores are present in the glomerular wall. Its higher excretion value was reported in type2 DM as compared to type1 DM patients in a study as compared to healthy subjects(Al-Malki, 2014). In another study, the decline in kidney function was observed with high urinary excretion of IgM showing inverse relation between IgM excretion and renal survival of type2 DM. IgM exretion is not considered an early marker for DN, yet validations are required for its consideration in clinical applicability(Cohen-Bucay & Viswanathan, 2012).

MCP-1
It is also known as Monocyte chemoattractant protein-1. Measurement of cytokines in urine may be helpful in diagnosis of diabetic kidney disease(DKD) as implicated in its pathogenesis. It was reported that the value of MCP-1/Creatinine in macroalbuminuric patients was higher as compared to normoalbuminuric patients and microalbuminuric patients. MCP-1 in urine was correlated with the rate of eGFR decline. MCP-1 was also reported high in patients with doubling of serum creatinine, in positive association with the risk of doubling serum creatinine as well as it remained independent predictors of doubling serum creatinine(Titan *et al*., 2012). Still studies are needed for investigation of role of urinary MCP-1 in normoalbuminuria and microalbuminuria in DKD.

TNF Alpha
Higher values of Urinary tumor Necrosis Factor alpha are reported in microalbuminuric and macroalbuminuric DM patients as compared to normoalbuminuric patients and their value is correlated with urinary NAG.A study of type 1 DM, it was found that the levels of IL6 and IL8, RANTES and Platelet derived growth factor are not changed in patients with normoalbuminuria but they show a higher value in case of microalbuminuria and they may have role in DN in patients with type 1 DM(Har *et al.*, 2013).While in case of type2 DM patients showed high value of IL8 in early stages of DN. Another study has reported high values of IL8, MCP-1, G-CSF, EOTAXIN, IP10 and RANTES in microalbuminuria patients as compared to normoalbuminuria or controls, hence their level monitoring might be useful in diagnosis of DN(Liu *et al.*, 2010).

VEGF

Vascular endothelial growth factor is produced by the podocytes at nephron level. It is considered as a podocyte biomarker. In type 2 diabetes patients VEGF is related to alpha1 microglobulin which is biomarker of proximal tubular lesions(Petrica *et al*., 2014). Higher values of VEGF than normoalbuminuria were reported by Kim *et al.* With increased values in patients having microalbuminuria and macroalbuminuria(Kim *et al.*, 2004).

Acute phase protein

Low grade inflammation in diabetic patients is indicated by the serum concentrations of acute phase proteins likeorsomucoid, CRP and fibrinogen. Levels of CRP and Fibrinogen have not been studied in relation to diabetes but their elevated circulatory levels have been observed in type 2 DM patients.On other hand serum and urinary levels of Orsomucoid are reported to elevate in type2 DM patients (even in normoalbuminuric patients) as compared to controls.

Orsomucoid

Orosomucoid are glycoprotein also called Alpha-1-glycoprotein of 41kDalton synthesized by empathic cells involved in inflammation as a regulatory protein. In normal condition its excretion is very low. In type 1 DM patients orsomucoid levels are high as compared to normoalbuminuric patients and controls.Increase of these values has been seen in patients with microalbuminuria and macroalbuminuria. In case of type 2 DM urine excretion of orsomucoid increases parallel to IgG, Ceruplasmin and transferring.Orsomucoid is considered an early biomarker of renal injury and significant independent marker of diabetic microvascular complications(El-Beblawy *et al*., 2016). Elevatedorsomucoid urinary excretion predictscardiovascular mortality with 39% of patients with normal UAE.So, urinary orsomucoid estimation might be an important marker of endothelial dysfunction, showing evolution of CVD in type 2 DM before onset of microalbuminuria.

OXIDATIVE STRESS MARKERS

8-Hydroxy-2'Deoxyguanosine

8-OHdG is an emerging biomarker of oxidative stress and DNA damage. It is excreted in urineand serves as marker of oxidative stress. Its independent increased value is reported in type2 DM patients as compared to control subjects. Its excretion in type 2 DM increases in both microvascular and macrovascular complications associated with diabetes. Microalbuminuric patients present a more higher value of 8-OHdG as compared to normoalbuminuric patients and its excretion is correlated to severity of disease and suggesting it as an effective early marker of stress during renal dysfunction.

Pentosidine

Pentosidine is the endproductof condensation of arginine and lysine protein residues with ribose. It is a marker of glycoxidation referring towardsoxidative stress. It accumulation increases with age but is more rapid in patients with diabetes. Its excretion in urine indicates tissue damage due to oxidative stress in DM patients.Urinary pentosidine level of type2 DM patients was significantlyhigh as compared to controls where its levels are correlated to glycemic control.An increase inpentosidine was also observed in type2 DM patients with diabetic nephropathy while its correlation with β2M was also indicated in another study. But it was not correlated to the current gold status of albuminuria(Daimon *et al.*, 2003). Hence, it is regarded as a useful biomarker of hyperglycemia- induced tissue damage in DM patients.

AGE

Commonly known as Urinary Advanced Glycation End Products (AGE) are eliminated in urine produce a toxic effect on tubules resulting tubular dysfunction. AGE values were high when compared to other biomarkers including urinary alpha-1-microglobulin and of urinary KIM-1 in

DM patients with mormaloalbuminuria(Petrica *et al*., 2015).Turk *et al*. found that 50% patients with normaloalbuminuria and 85% patients with microalbuminuria in type 2 DM have high values of urinary AGE(Turk *et al.*, 2004).

Intrarenal RAAS System

Angiotensinogen

Angiotensinogen is synthesized by the proximal tubule cells and it is converted into Angiotensin I by Renin and then to AngiotensinII by Angiotensin converting enzyme(ACE)(Satirapoj *et al.*, 2014). As the elements of RAAS are present on kidney levels and are involved in activation of RAAS in DM patients hence their presence may be reported in pathogenesis of DN.In type1 DM patients urinary angiotensinogen excretion value is high prior to microalbuminuria predicting role in normotensive type 1 DM(Zhuang *et al.*, 2015).In type2 DM urinary angiotensinogen shows increased level of excretion in micro- and macroalbuminuric patients as compared to controls while the normoalbuminuria patients also have a higher excretion value as compared to controls representing its significance as a marker of injury in both albuminuric and non-albuminuric patients(Satirapoj *et al.*, 2014).

Its association with other biomarkers is also observed. In correlation to α-1-microglobulin it indicates intrarenal RAAS activated tubular injury in type2 DM patients. A correlation between urinary ACR and beta-2 microglobulin indicates the decline of renal functionand cardiovascular complications in type 2 DM patients with DN and high values of angiotensinogen. Increased ACE activity may also serve as a marker of albuminuric and non-albuminuric type2 DM patients as compared to healthy subjects. More researches are required for the identification and validity of angiotensinogen as a marker of type 2 DN(Mizushige *et al*., 2015).

VASOCONSTRICTION BIOMARKERS

Thromboxane

Thromboxane A2 plays role as a vasoconstrictor as well as platelet activator which is synthesized by platelets and renal cells. It is metabolized into thromboxane in blood where it is further metabolized to 2,3-dinor-TXB2 reflecting platelet synthesis (after urinary excretion) and in urine which reflects renal TXB2. Hypercoagulability and platelet hyperactivity has been conformed clinically in patients with type 2 diabetes that showed thromboxane excretion in urine in comparison to controls even when mild protenuria is absent but not with severe protenuria(Okumura *et al.*, 2003).Katayama *et al*. suggested increase renal production of TXA2 with platelet activation effecting the renal function of type 2 diabetic patients(Seligman *et al.*,2000).Prostacyclin is an endothelial protein having anti-aggregating properties.

High TXA2/PGI2 ratio has been observed red in type 2 DM patients exhibiting retinopathy. So, by monitoring this ratio detection of diabetic vascular complications may be facilitated(Hishinuma *et al.*,2001). TXB2 excretion may reflect pathological changes associated with retinopathy and nephropathy during type 2diabetes but it still has to be compared with UAE performance.

Endothelin 1

It is an important vasoconstrictor synthesized as a peptide by the endothelium. Its urine concentration is high while its plasma concentration is very low. It is produced by mesangial cells, glomerular and tubular epithelial cells(Seligman *et al.*, 2000). Elevated levels of ET-1 in type 2diabetes were first reported by Shin *et al.* in 1999 but later and concurrent studies did not found any associated significant increase in DM patients. There is a contradiction in the reported result about the link between DM and ET-1 hence further study seems necessary.

PROTEOMIC BIOMARKER

Proteome

Protein separation through capillary electrophoresis and later its mass spectrometric analysis has enabled to search new potential biomarkers by urinary proteome analysis facilitating the diagnosis and progression study of diseases. This approach has reported new urinary biomarkers for DN and also differentiate between type 1 and type 2 DM biomarker(Zürbig *et al.*, 2012). Proteomics revealed that microalbuminuria is not a perfect biomarker of DN, while it was reported that collagen fragments were significant biomarker even 3-5years prior to the onset of microalbuminuria(Demir *et al.*, 2012). 3 proteins of urinary exosome are also present differentially in urine of DN patients(Zubiri *et al.*, 2014). In another study Alpha-2 glycoprotein, Alpha-1 acid glycoprotein, Alpha-1 microglobulin and IgG were found accompanying with albumin in urine samples of type2 DM(Zürbig *et al*., 2012).

Proteomic study has identified distinctive biomarlers for albuminuria, Diabetes, allowed early diagnosis of DN, difference between non-diabetic kidney diseases predicted development of DN from normoalbuminuric DM patients(Caseiro *et al.*, 2014). More investigations and further study is required for discovery of new markers of DM and DN at early stages but this field has to face limitations as this technology is expensive and available in only few labs.

H-FABP

Heart fattyacid binding protein isa marker of distal tubulardamage. Uni-nephrectomized diabetic rats indicated the appearance of H-FABP before albuminuria and urinary protein excretion.Nauta*et al.* in a cohort study of type 1 and 2 DM patients with biomarkers of glomerular lesions (IgG) , proximal tubular lesions (KIM-1, NAG, NGAL and Cystatin) and distal tubular lesions (H-FABP) in relation to UAE and GFR reported higher values of NAG, NGAL and H-FABP in normoalmuninuria than control patients while value of Cystatin C was low showing mormoalbuminuric DM patients with both proximal and distal tubular lesions(Nauta *et al*., 2011).

Zinc AlphaGlucoprotein

Zinc alpha glycoprotein has been indicated as a novel urinary biomarker of non-albuminuric DN in males with type 2 Diabetes(Lim *et al*., 2012).

Liver type fatty acid binding protein

L-FABP is a low molecular weight (15kD) protein produced in the proximal tubular cells cytoplasm(Kamijo-Ikemori *et al.*, 2011). Serum L-FABP has no effect on urinary L-FABP suggesting that the L-FABP in urine is an indication of tubulo-intestinal injury, representing stress on proximal tubules(Kamijo *et al.*, 2006). High levels of L-FABP are seen in diabetic patients as compared to controls.In type1 DM normoalbuminuric patients, high L-FABP level predicts progression towards microalbuminuria and macroalbuminuria.Normoalbuminuric patients of type2 DM also showed increased levels of L-FABP suggesting it as a useful marker of DN infact Ministry of Health and Welfare in Japan has confirmed it. L-FABP levels are also high in microalbuminuric stage. Its values increase with the decrease of renal function related to the severity of disease. A study also considered it as a independent marker of DN as it has a correlation to blood pressure(systolic), fasting blood glucose and HbA1c(Viswanathan *et al*., 2015).

Ceruloplasmin

It is a copper carrying serum protein with a molecular weight of 151kD and it is highly negatively charged than albumin and hence it is not easy for glomerulus to filter it. Its presence is reported in type 2 DM patients with normoalbuminuria and increase in urine predicts development of microalbuminuria, indicating it a biomarker even before albuminuria as Transferrin and IgG. The values of Transferrin, IgG and Ceruloplasmin increase parallel in type2

DM(Nauta *et al*., 2011). 90-91% sensitivity and 61-66% specificity is shown by ceruloplasmincretanine ratio of diabetic and non-diabetic DN patients. Further study is required to study use of ceruloplasmin as a biomarker and its comparison with albuminuria.

Urinary Micro RNA

Recently, micro RNA has emerged as an important class of short non coding RNA as post-transcriptional regulators of gene expression, with ability of regulating numerous biological functions including focus on the role of mi RNAs as mediators or biomarkers of diseases, including diabetes. Identification of many mi RNAs in body serums and fluids has been reported which by up regulation or down regulation may be involved in progression of DN. Thus their detection on early stages may be valuable topredict the disease. Differentially expressed 27 mi RNAs in urine samples of type 1 diabetes were reported by Argyropoulos*et al*(2013). Further studies are required on large population with diabetic nephropathy to understand the mi RNA as DN biomarker.

CONCLUSION

The increased excretion of urinary biomarkers in diabetes indicates the presence of renal dysfunction. The marker of Glomerular damage Transferrin, podocyte injury, nephrinuria and L-PGDS excretion can be related to renal complication detection in diabetes. TypeIV collagen, Fibronection, NAG, NGAL and KIM-1 molecules have also predicted their roles in the diagnostic and disease prognosis pathway even before albuminuria. Oxidative stress markers (8OHdG and pentosidine) and vasoconstrictors (Endothelin-1 and Thromboxine) are also presented in literature showing significant results. Low grade inflammation is indicated by the release of different inflammatory molecules such as cytokines, Antibodies such as IgG and IgM and other molecules. Study of renal proteomics and urinary microRNA studies are new emerging fields and they require more study for their clinical application. In-short more sensitive and specific markers of diabetic renal complications are present but their efficiency as compared to Albuminuria is yet to be tested for their economical use in diagnostics.

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