

Diabetic nephropathy: Pathogenesis and therapeutic management

Nafisa Saleem¹, Muhammad Naeem¹, Abid Rashid², Naheed Akhter¹, Imtiaz Mahmood Tahir¹, Mohsin Khurshid^{1*}

Abstract: Nephropathy is considered a foremost cause of morbidity and mortality in patients with type 1 or type 2 diabetes mellitus. Hyperglycemia causes renal injury directly or via hemodynamic alterations. These alterations induce glomerular hyperfiltration, microalbuminuria, mesangial expansion, and GBM thickening and arteriolar hyalinosis. Risk determination has potential importance in the management of Diabetic Neuropathy advancement. In addition to the traditional approaches through albuminuria and glomerular filtration rate for the prediction and monitoring of the rate of damage among diabetic patients, various studies are enduring to identify biomarkers. Recent approaches to treat this disorder emphasize on increased control of glycemia and blood pressure using therapies based on renin-angiotensin-aldosterone system blockade. Renal and pancreatic transplantation is considered the best modality. This review of literature focuses on pathogenic factors, risk factors, diagnosis and treatment of diabetic nephropathy.

¹ College of Allied Health Professionals, Directorate of Medical Sciences, Government College University Faisalabad, Pakistan

² Directorate of Medical Sciences Government College University, Faisalabad.

Corresponding author email:
mohsinkhurshid@gcuf.edu.pk

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INTRODUCTION

The plasma volume, the levels of extracellular fluids, pH and salt levels are mainly controlled by the kidney and it is the basic function of the kidneys (1). One of a recognized complication of diabetes is Diabetic Nephropathy, responsible for mortality and morbidity and hence leads to End Stage Renal Failure (ESRD) (2). Diabetic nephropathy (DN) refers to a characteristic set of structural and functional kidney abnormalities in patients with diabetes (3). Diabetic Nephropathy clinical aspects involve raised creatinine levels, proteinuria and then eventually low glomerular filtration rate (GFR) (4). Renal disease clinical features in diabetic patients include renal dysfunction, increase in urinary albumin excretion with patients at risk going through the stages of normoalbuminuria, microalbuminuria, overt proteinuria, and finally end-stage renal disease (ESRD) (5). The rapid decrease in glomerular filtration rate (GFR) comprised of inflammation and tubular injury, necrosis, impaired microcirculation and decreased renal blood flow which leads to acute renal disease (6).

The stages of Diabetic nephropathy in both type 1 and type 2 diabetes continue from

normoalbuminuria to microalbuminuria and macro-albuminuria in somewhat expected fashion, that indicates raising creatinine level as the concluding clinical index of overt diabetic nephropathy. In type 2 diabetic patients, this strategic classification has a sound effect on mortality with per year death rates of 0.7%, 2.0%, 3.5% and 12.1% for normoalbuminuria, microalbuminuria, macro-albuminuria and increasing creatinine level (7). Around 13 % adults are affected by Chronic Kidney Disease (CKD) whereas 1-2 % Patients of CKD requiring kidney replacement over a period of 10 years only progresses to ESRD, the majority of patients do not develop ESRD (8). In type 2 diabetic patients screening for microalbuminuria should be done at the onset of disease while in type 1 diabetic patients screening for microalbuminuria should be performed after 5 years of disease duration as micro-albuminuria within a short duration of disease occurs rarely. There is some evidence that pre-pubescent period of the disease is more important in microvascular complications development; hence, before following such recommendations, clinical assessments should be performed (9). Tubular damage markers

detection in urine is crucial in the early period of DN and confers to DN development primarily rather than in a secondary way. Detection of these tubular damage markers in urinary excretion could be beneficial in the assessment of damage to renal tubules in the initial stage of potential diabetic nephropathy (DN) (10).

Structural changes in kidneys comprised of increased extracellular matrix accumulation in mesangium, and GBM thickening cause damage to the above parameters. Afterward, mesangial nodules formation occur which exemplifies the distinctive lesions of diabetic nephropathy along with tubulointerstitial lesions (11). Experimental studies have evolved several new modalities of treatments based on diabetic nephropathy pathogenic factors, comprising of strict glycemic and BP control, renin-angiotensin-aldosterone system (RAAS) blockade with ARB and ACE inhibitors and improvements in lifestyle e.g. dietary limitations, exercise and other new means (12).

Pathogenesis

The basic mediators of renal damage involve hemodynamic and metabolic stimuli provoked through hyperglycemia (13). Structural and functional changes such as increased ECM deposition and raised GBM permeability in renal cells are induced by hyperglycemia to produce cytokines, growth factors and humoral mediators (11). These alterations stimulate ischemic, inflammatory, fibrotic and pro-oxidant pathways resulting in thinning and loss of podocyte, GBM thickening, tubular atrophy, tubulo-interstitial inflammation, kidney arteriolar hyalinosis and mesangial matrix deposition (14). Such structural and functional changes headed towards diabetic nephropathy (11).

Hemodynamic Factors

Hemodynamic factors include actuation of vasoactive hormones, exacerbation of glomerular pressure and systemic pressure involving vascular endothelial growth factor (VEGF), endothelin and renin-angiotensin-aldosterone system (RAAS) which contribute to Diabetic Nephropathy. Hemodynamic alterations present initially in the disease course perform a crucial

role, increasing albumin passage through glomerular capillaries, conferring to podocyte injury, nephron loss and mesangial matrix accumulation (15). Hemodynamic dysfunctions are presented as glomerular hypertension, blood arterial hypertension and hyperfiltration in diabetic patients (16).

It is demonstrated that to minimize a hemodynamic dysfunction, the involuntary stretch is applied which stimulates glucose transporter, GLUT-1 over expression and produce Transforming Growth Factors beta-1, (TGF beta-1) in mesangial cells of rats. Monoclonal anti-TGF-1 antibody reduces GLUT-1 over expression and hence intracellular transport of glucose *in vitro*. Involuntary stretch in diabetic patients is responsible for exacerbation permeability of glomeruli to proteins. Vascular permeability factor (VPF) is the potent advocator of this abnormality (17). Glucose influx is induced by GLUT-1 in renal cells, a surface receptor of renal cells. It is demonstrated that GLUT-1 mRNA over expression and GLUT-1 proteins overproduction induce glucose influx (23-30nM), *in vitro*, in mesangial cells. Moreover, the GLUT-1 expression is stimulated by TGF-beta 1 and exacerbation of glucose transport occur in cells (18).

Metabolic Factors

During chronic hyperglycemia, a combination of glucose and free amino acids on tissue proteins occurs. In this non- enzymatic phenomenon, initial reversible glycosylation products are produced early and later irreversible advanced glycosylation end products (AGEs) are formed. Specific receptors are actuated by AGE's initializing cellular dysfunction and damage. In particular properties of podocyte slit membrane, a functional abnormality related to AGE may help in the progression of albuminuria. Elevated levels of AGE are due to hyperglycemia in diabetic patients. Advanced glycosylation end products induce intrinsic glomerular cells to generate TGF-1 confers to tubulointerstitial injury and glomerulosclerosis via abnormal production of ECM (11).

Oxidative Stress/Inflammation

Reactive oxygen species (ROS) over production

induced via hyperglycemia can be applicable in diabetic complications pathology (19). It has been experimentally demonstrated that DN may be well attributed to less production of mitochondrial superoxide and such elevated production might debilitate diabetic nephropathy (20). The primary conciliators of diabetic nephropathy are metabolic pathways. Metabolic pathways cause actuation of the immune system and chronic inflammation. It is observed in several studies that elevation in glomeruli in monocytes or macrophages convincingly confer to the progression of diabetic nephropathy. Intrinsic cell of the kidney, involving renal tubular cells, mesangial, glomerular endothelial and dendritic cells can produce growth factors and inflammatory cytokines such as TGF- β , interleukin 1(IL-1), IL-18, IL-6 along with tumor necrosis factor-alpha (TNF-alpha) and VEGF all have been involved in the progression of diabetic nephropathy. Accumulation of matrix proteins is the major causation of the development of renal injury in DN which can be due to low degradation or increased synthesis of matrix proteins (5). A crucial risk factor for the progression of DN is hyperglycemia. Abnormal actuation of protein kinase C (PKC) is induced by hyperglycemia, which in turn is involved in the progression of DN. This PKC upregulation has been observed in rats' kidneys with DN. It is linked with the upregulation of collagen type IV, fibronectin and TGF- β 1(21).

Renal Pathology

Pathological changes in kidneys occurs chronic diabetic patients rather than before the commencement of microalbuminuria (22). That light microscopic examination revealed diabetic nephropathy contain three main lesions thickened GBM, and tubular basement membranes, diffuse mesangial accumulation, thickened tubular basement membrane, and glomerular basement membrane and afferent and efferent arterioles hyalinosis. In latest classification, Class I comprise of GBM thickening confirmed by electron microscopy, regulated accordingly for age and gender. Class II contains mild (IIA) to severe (IIB) mesangial expansion. First alterations that take place in diabetes at 2-5 years are thickening of

GBM and mesangial matrix accumulation (23). Class III comprises lesion, nodular glomeruli sclerosis, first explained in 1936 by Kimmelstiel and Wilson. Finally, Class IV is consisting of global glomeruli sclerosis >50% together with further Classes I, II, or III lesions. Tubulointerstitial inflammation, vascular lesions, and atrophy are ranked from 0-2 or 0-3 individually. Adjacent to the epithelial parietal surface of Bowman's capsule, arteriosclerosis, glomerular capillary sub-endothelial hyaline, arteriolar hyalinosis and capsular drops (also called exudative lesions of diabetic nephropathy) also might be present. Several associations among tubulointerstitial alterations and functional results have been reported. Interstitial fibrosis is frequently relative to tubular atrophy, a potential predictor of the evolution from moderate to severe GFR decline (23).

RISK FACTORS

Increased urinary albumin excretion

In type 1 and type 2 diabetes, increased excretion of urinary albumin is an important risk factor in the development of DN. Elevated UAE e.g. 30-300mg/g creatinine so-called microalbuminuria is the first intimation of diabetic nephropathy in spot urine sample in many of patients (24). In patients with severely developed elevated albuminuria (>300mg albumin/g creatinine) are specifically at a higher risk for renal function decline in spot urine sample also referred to as microalbuminuria (25). Only 40% of patients with moderate albuminuria return back to normoalbuminuria (26). Moreover, in T1D and T2D, almost 50% of patients undergo GFR decline, even in the presence of normoalbuminuria or moderate albuminuria (27).

Elevated glucose level

Poor glycemic control is the major risk factor in the development of diabetic nephropathy. Observational studies suggested that adequate glycemic control in T1D and T2D patients induce a decline in the DN incidence (28). Moreover, advancement from severe albuminuria to decreased GFR or to ESRD can be reduced through severe glycemic control(29).

Dyslipidemia

It is observed in DCCT/EDIC study, low risk of advancement of moderate to severe albuminuria or to ESRD is linked with low levels of (TG) triglycerides and (LDL-C) low-density lipoprotein cholesterol and hence, Dyslipidemia is important in the DN pathogenesis. Raised risk of progression of elevated UAE, moderate or severe, is also linked with the high level of (TC) total cholesterol in type 2 diabetic patients (30).

Others

An important risk factor for chronic diabetic nephropathy is longer duration diabetic patients (31). Another significant risk factor of developing nephropathy is increased blood pressure (32). It is observed in a study of DCCT/EDIC, lower risk of advancement from moderate to severe albuminuria and to ESRD is linked with low BP (30). Moreover, in type 2 diabetic patients, retrogradation of moderate to normoalbuminuria is linked with low BP (33). Another factor linked to elevated risk for diabetic nephropathy is obesity (34). The DCCT study suggested that obesity, derived from waist circumference, is not associated with GFR decline but with increased albuminuria occurrence (35).

Table 1: Diabetic Nephropathy; Modifiable and non-modifiable risk factors

Modifiable risk factor	Non-modifiable risk factor
Increased urinary albumin excretion	Longer duration of disease
Elevated glucose levels	Advanced age
Elevated blood pressure	Female sex
Dyslipidemia	
Obesity	

DIAGNOSTIC CRITERIA

Diagnosis and monitoring of diabetic nephropathy include two approaches based on pathogenesis, evaluation of renal damage as albuminuria and estimation of renal function glomerular filtration rate (36). Proteinuria and alterations in serum creatinine, indicative of GFR decline are diagnostics of diabetic nephropathy in clinical

practice (37).

Screening for albuminuria

In T1D patients, screening should start after the onset of diabetes, 5 years duration of disease. In T2D patients, it should be done at the time of identification. Urine albumin/creatinine ratio is the most favored screening test, collected as the first morning voided spot sample. If it detects microalbuminuria, confirmation for constancy must be done within 6 months, following 2 to 3 repeated positive estimations. Methods of screening for microalbuminuria can be (1) Estimation of albumin/creatinine ratio in random spot collection. (2) Measurement of creatinine along with creatinine clearance (24-h collection). Timed collection 4-h or overnight (38).

Urinary albumin excretion (UAE) rate for microalbuminuria is 20–200 µg/min (30–300 mg per day) and albumin/creatinine ratio of 2.5–25.0 mg/mmol for males, 3.5–35.0 mg/mmol for females (39).

Estimated Glomerular Filtration Rate

GFR declines after renal structural injury and other renal functions also reduced along with GFR in CKD and hence are the only and best-accepted constituent of renal excretory function. GFR is estimated via serum creatinine (40). Renal function abnormality is graded according to GFR five classes (41).

Biomarkers

Primary biomarker for diabetic nephropathy can be urinary angiotensinogen (42). In patients with type 2 diabetic normoalbuminuria, urinary angiotensinogen is elevated than normal. In patients with microalbuminuria and macroalbuminuria, there is a progressive increase in urinary angiotensinogen (43).

At low levels of proteinuria, albumin shows much variability than at advanced levels. Consequently, it is proposed that a significant biomarker for early diagnosis of progression of DN, instead of albuminuria could be non-albumin proteinuria (NAP) (44, 45). Urinary NAP involves several urinary proteins, some of them, comprising immunoglobulin G (IgG), α-1 microglobulin, β-2 microglobulin, Cystatin C,

transferrin, metalloproteinase-9, tissue inhibitors of metalloproteinases-1, nephrin are linked with renal injury (46).

Above all, elevated levels of β -2 microglobulin and ubiquitin are associated with the advancement of diabetic nephropathy (47). Cystatin C, a pivotal identified biomarker of kidney failure, metabolized through proximal tubule and filtered freely via renal glomeruli is a cysteine protease inhibitor (48). In differentiating T2D patients with decreased GFR from patients with control GFR, Cystatin C is a more precise serum biomarker compared to Cockcroft and Gault eGFR or serum creatinine (49). Though such tests are apposite for advanced diabetic nephropathy patients, at early clinical phases of the disease, high variability among individuals and so moderate

sensitivity and specificity confines early diagnosis of disease with these customary tests (50).

Studies conducted to estimate vital proteins in urine or serum appear to be associated with many clinical pathological processes involved in the vulnerability and development of diabetic nephropathy. For instance, in DN, transforming growth factor β (TGF- β) is regarded as a significant contributor to fibrosis (51). In an ADVANCED cohort study, for ESRD prediction, a potential biomarker was assayed in patients who all had T2DM. ESRD developed in a minority of patients in this large study who developed ESRD. Before the commencement of ESRD, in plasma, profibrotic factor TGF- β 1 and the antifibrotic factor bone morphogenetic protein 7 (BMP-7) were estimated. There was a positive association

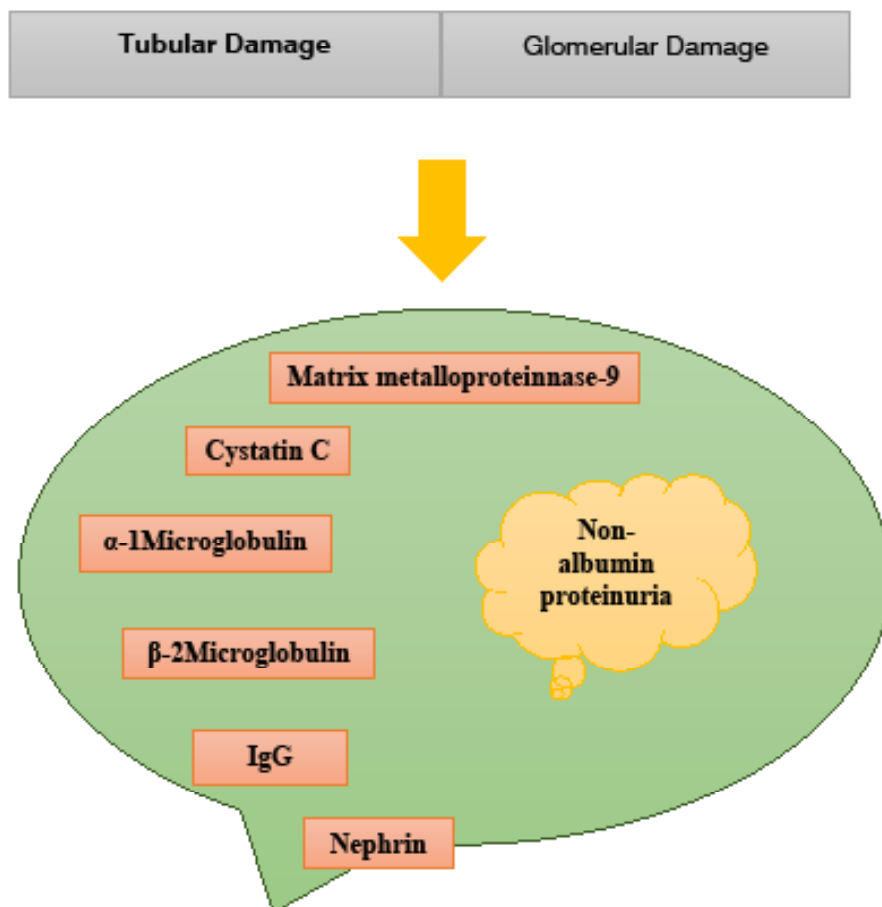


Figure 1: Pathogenesis of Diabetic nephropathy

of elevated risk of DN with TGF- β 1: BMP-7 ratio.

Addition of this ratio to UAE headed towards increased ESRD prediction (52, 53). In a study, 156 T1DM patients were evaluated for prediction of DN by the estimation of proteins, highly sensitive C-reactive protein and mannose-binding protein C (MBP-C) (54). Highly sensitive C-reactive protein and MBP-C, baseline levels of both proteins elevate with elevated albuminuria levels. Another cohort study suggested that MBP-C values confirm the preceding findings (55). In the previous few years, in T1D and T2D patients, soluble TNF receptors 1 and 2 and TNF α receptors, circulating levels of these inflammatory markers found to predict renal function deterioration (56, 57).

TREATMENT

Glycemic Control

The treatment of diabetes including glycemic control, lipid control, decrease in blood hypertension and eradicating smoking. As renal structural changes are due to hyperglycemia, therefore, in patients with higher risk of progression to diabetic nephropathy, glycemic control remains the key target of treatment. To reduce the risk of microvascular complications strict glycemic control is the best line of approach. Moreover, because of raised life expectancy, early therapy with glycemic control in young diabetic patients has spectacular results on their survival (58, 59). The onset of new microalbuminuria and early glomerular hyperfiltration can be inverse to some extent by glycemic control. Many observational studies have shown a close association between diminished glycemic control, microvascular complications and diabetic nephropathy (60).

As compared to the proof of strict glycemic control on macrovascular complications, in randomized controlled trials, severe glycemic control is constructive in the onset and development of DN delay (61, 62). It is verified in two reports that neuropathy, cardiovascular disease, retinopathy and predominantly diabetic nephropathy (50%) decrease following strict glycemic control with inulin or sulfonylureas. Therefore, recent strategies proposed a targeted HbA1c of \sim 7.0% (53 mmol/mol) to inhibit or to delay the evolution of diabetic

microvascular complications and diabetic renal disease (40, 63). Decreased risk for microvascular and cardiovascular complications is almost 50%, verified in a multifactorial intervention study (64).

Blood Pressure Control

Primary management of hypertension in both type 1 and type 2 diabetic patients play significant role in treatment of diabetic nephropathy (39). Up till now, a standard blood pressure of <130 mmHg systolic and <80 mmHg diastolic has been setup by the ADA National Kidney Foundation (66). Based on recent trials, the Eighth Joint National Committee recommended a blood pressure of 140/90 mm Hg for all patients with diabetes mellitus or CKD (66).

In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, though strict control of blood pressure decreases A3 class albuminuria but more often in severe blood pressure arm adverse problems were also experienced (67). Specifically, in patients with albuminuria, 130/80 mm Hg is thought to be the standard BP (68). It is studied in Irbesartan Diabetic Nephropathy Trial (IDNT), progressive decrease in systolic BP to 120 mmHg is linked with enhanced kidney and patient survival unaffected by the basic functions of the kidneys (69). For patients with diabetes with or/and without UAE (>30 mg/d) thresholds have been suggested for controlling BP in CKD to begin treatment of lowering blood pressure of 130/80 mmHg and 140/0 mmHg by KDIGO clinical practice guideline (70).

Protein Restriction

In CKD, the function of nutritive protein restriction is debatable (71). In a randomized trial, T1D and overt nephropathy patients were involved and lower BP with stable kidney function is compared with increased intake of phosphorous and protein. Protein restriction (0.6g of protein/kg of body weight/day) and phosphorous (500mg-1g/day) showed decrease in GFR decline. Moreover, in another study in T1D patients, protein restriction (0.8g/kg body weight/day) that is constant with the daily allocated recommendations, decrease advancement towards ESRD (72). There are recommendations by the National Kidney Foundation that there should be an intake of

protein 0.6g/kg body weight in patients with GFR <29 mL/min per 1.73m² every day (73).

Cessation of Smoking

In type 2 diabetic patients, the threat of advancement is decreased by 30% by the cessation of smoking alone. There is a gradual loss of renal function in people who terminate smoking (74).

RAAS Blockade with ARB's, ACE and DRI

The renin- angiotensin- aldosterone system (RAAS) has the main function of regulating blood pressure and sodium homeostasis. The key effect or of RAAS, angiotensin II (Ang II) by interaction with angiotensin type 1 (AT1) and type 2 (AT2) receptors increases the vascular tone of efferent and afferent glomerular arterioles regulating intra-glomerular pressure. In addition to the hemodynamic effects, AT1 receptor activation activates expression and release of a variety of profibrotic and proinflammatory mediators involved in the development of diabetic nephropathy.

Angiotensin receptor blockers (ARBs) or angiotensin-converting enzyme inhibitors (ACEi) are the foremost effective antihypertensive agent that decreases the advancement of diabetic nephropathy in diabetic patients with hypertension (5). To control diabetes administration of ARBs and ACEi in patients with moderately raised albuminuria is recommended and in those with highly elevated albuminuria A3 and eGFR (<60 mL/min/1.73m²), is highly recommended (75). In 2002, a double-blind randomized clinical study used 2 (ACEi) angiotensin-converting enzyme inhibitors, captopril, imidapril, and placebo. (Japan-IDDM: Japanese trial of ACE inhibitors on renal protection against nephropathy in type 1 diabetes). Imidapril is a pro drug of imidaprilat and is deprived of sulfhydryl radical. Imidapril has two-fold high effectiveness than captopril in preventing activity of ACE and has the equivalent effect as an active form of enalapril (76). In T1D patients, elevation in UAE in microalbuminuria and macro-albuminuria is inhibited by captopril and imidapril and the blood pressure aimed may be less than 130/80 mmHg (76).

In 2011, an ARB, olmesartan effects were examined on initial consequences of a two fold increase in serum creatinine and ESRD and death in overt nephropathy patients in China and Japan. Blood pressure, the frequency of alteration in reciprocal serum creatinine and proteinuria potentially reduced by olmesartan. It is determined that olmesartan did not increase the results above the ACEi but it was tolerated well (77). Combined treatment in diabetic nephropathy with ARB, losartan and ACE inhibitor, Lisinopril reduces proteinuria but its effect on the advancement of the renal disease and its safety report is ambiguous. It is determined that a combined treatment with ARB and ACE inhibitor is linked with the raised threat of adversative events in T2DN patients in USA (78).

Combined therapy with a direct renin inhibitor, aliskiren, and losartan, an ARB possesses renoprotection which is free from effects of lower blood pressure in T2DN patients. In comparison to placebo, the daily dosage of aliskiren (300 mg) decreases 20% of average UAE in 24.7% patients by 50% or more in relation to patients (12.5%) who have received placebo (79). Also in two clinical trials, combined treatment with ARBs and ACEi are linked with increased frequency of adversative events, furthermore, no fruitful effects were seen in diabetic nephropathy and CVD (78, 80).

Renal Replacement Therapies

Different approaches for ESRD patients with diabetes such as hemodialysis, peritoneal dialysis, and kidney transplantation are present. Several studies revealed analogous survival with peritoneal and hemodialysis, although patients survival is more associated with hemodialysis than peritoneal dialysis. Sexual activity, communal life and leisureliness of patients are bounded by both peritoneal and hemodialysis. In comparison to dialysis, renal transplantation is linked to enhanced survival, better condition of life and a greater degree of rehabilitation (81).

Renal transplantation is successful in younger patients having no cardiac disorders. In allografts, diabetic nephropathy recurrence can happen which is the outcome of deficiency of insulin and

poor glycemic control. Pancreas transplantation is considered to be the best modality in case of renal lesions in DN. Serial renal biopsy study verified that after pancreas transplantation, mesangial volume, GBM thickness, and mesangial matrix steadily decreases after 5-10 years.

Novel Therapies

MCP-1, a protein secreted that attracts macrophages and monocytes particularly through its cell surface receptor, CCR2 towards its origin. Renal cells respond to various proinflammatory signals and produce MCP-1 (82). Certainly, the MCP-1 expression is recognized in renal diseases including potential inflammation involving DN MCP-1 mRNA formation and release of protein via cultured kidney parenchymal cells is induced by constituents of diabetic milieu, dignifying that diabetic onset can induce recruitment of macrophages (82, 83).

Although the outlook for diabetic nephropathy has improved over the past few decades with better glycemic and blood pressure control, there remains a significant unmet need. Many innovative approaches have been proposed newly that

indirectly decrease renal expression of MCP-1 and in animal models by pointing downstream intracellular signaling pathways or diabetic renal inflammation, persuaded via hyperglycemia. It involves usage of PRAR agonist, an analog of 1, 25-dihydroxyvitamin D3, flavonoid, PKC-inhibitor and eicosatetraenoic acid (82).

CONCLUSION

Though the DN pathogenesis includes both environmental and genomic aspects, the genes responsible for the commencement and disease progression are still ambiguous. Investigators at present are concentrating on primary biomarkers to forecast the rate of renal damage beyond albuminuria. Moreover, combined innovative therapies and established traditional treatments may decrease the immense load linked to DN. Hopefully, with proliferating understanding on the diabetic nephropathy pathogenesis, novel therapies presently in preclinical or clinical progress will prove to be protective and in some instances, the inverse of diabetic nephropathy.

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