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Diabetic Glomerulopathy

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1. Introduction

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD) in adults. In the United States, almost half of patients entering ESRD programs were diabetic, and most of them (≥80%) had type 2 diabetes. This is due to the facts that 1) diabetes, particularly type 2, is increasing in prevalence; 2) diabetes patients now live longer; and 3) patients with diabetic ESRD are now being accepted for treatment in ESRD programs where formerly they had been excluded. The annual cost of caring for these patients, in the United States alone, exceeds $10 billion. The mortality rate of patients with diabetic nephropathy is high, and a marked increase in cardiovascular risk accounts for more than half of the increased mortality among these patients.

The earliest clinical manifestation of renal involvement in diabetes is an increase in albumin excretion (microalbuminuria), a stage termed as incipient nephropathy at which renal histology may be relatively normal or may reveal glomerulosclerosis. Diabetes can cause a variety of pathological abnormalities: isolated glomerular basement membrane thickening, mesangial expansion, nodular intercapillary and/or diffuse glomeruloscelorsis or even advanced diabetic sclerosis. While glomerulopathy is quite the hallmark of diabetic nephropathy, the frequency and functional significance of interstitial lesions in diabetic kidney is now well recognized. Furthermore, the occurrence of non-diabetic glomerulopathy or vasculitis alone or superimposed on diabetic nephropathy is increasingly being documented in literature. The pathogenesis is incompletely understood and is very vigorously being investigated.

Once overt diabetic nephropathy (proteinuria) is present, ESRD can be postponed, but in most instances not prevented, by effective antihypertensive treatment and careful glycemic control. Treatment options currently are limited to obvious pathogenic factors while several innovative therapies are under evaluation. Therefore, in the last decades, there has been intensive research into pathophysiologic mechanisms of early diabetic renal injury, predictors of diabetic nephropathy risk, and early intervention strategies

2. Epidemiology

Type 1 diabetes — The epidemiology of diabetic nephropathy has been best studied in patients with type 1 disease, since the time of clinical onset is usually known. About 0.5% of

the population in the United States and Central Europe has type 1 diabetes. The prevalence is higher in the northern Scandinavian countries and lower in southern Europe and Japan. Approximately 20 to 30 percent will have microalbuminuria after a mean duration of diabetes of 15 years (Orchard TJ 1990). Less than half of these patients will progress to overt nephropathy; microalbuminuria may regress or remain stable in a substantial proportion, probably related to glycemic and blood pressure control.

Prior to the current period of intensive monitoring and treatment, it was suggested that 25 to 45 percent of diabetic patients will develop clinically evident disease (the minimal criterion for which is a persistently positive urine dipstick for protein) (Parving HH, 1998). After so-called macroalbuminuria or clinical grade proteinuria (>300 mg albuminuria per day) develops, the majority of patients will progress to end-stage renal failure.
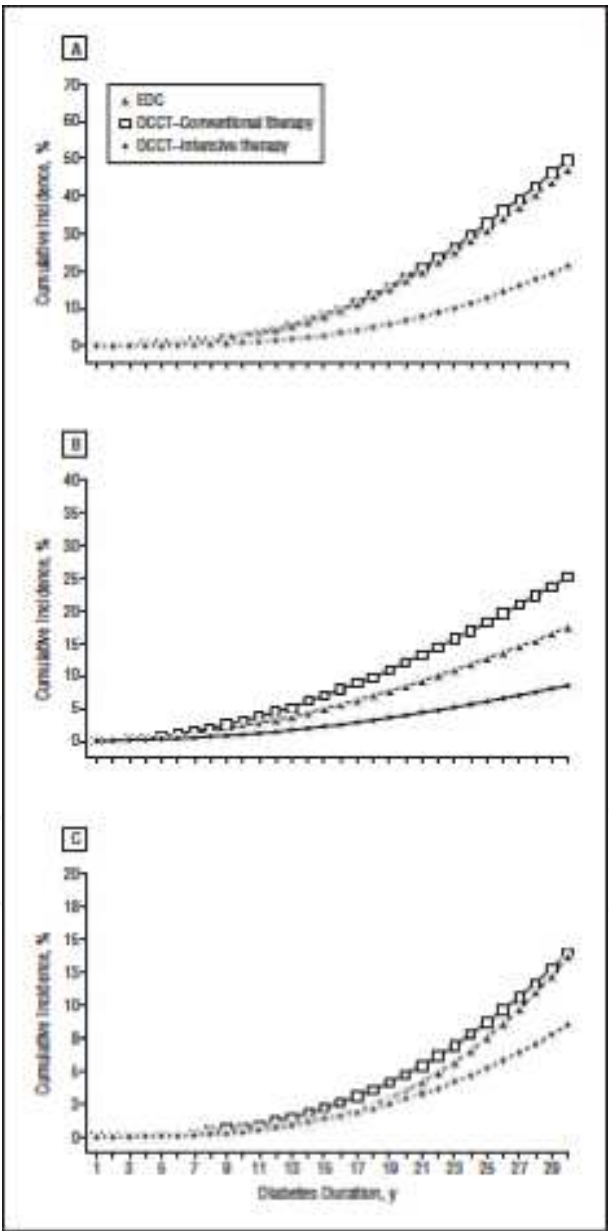

Fig. 1. Estimated cumulative incidences of proliferative retinopathy or worse (A), nephropathy (B) and cardiovascular disease over time.

The overall incidence of end-stage renal disease (ESRD) was also substantial, with reported rates of 4 to 17 percent at 20 years from time of initial diagnosis and approximately 16 percent at 30 years (Nathan DM 2009). A strong predictor of the development of ESRD was the level of glycemic control during the first two decades of IDDM. The risk of ESRD in the group with the poorest glycemic control was almost threefold higher than in the middle group, and fourfold higher than the group with the best glycemic control.

In comparison to these findings, subsequent studies have found that the renal prognosis of type 1 diabetes, including the rate of progression to ESRD, has dramatically improved over the last several decades. In addition to the importance of glycemic control, more aggressive blood pressure reduction and the use of angiotensin converting enzyme inhibitors have been shown to reduce the rate of progression of, though not prevent, diabetic nephropathy (Krolewski M 1996).

Type 2 diabetes is about nine times more prevalent than type 1 diabetes, accounting in part for the greater contribution of type 2 diabetic patients to ESRD incidence. In Caucasians, the prevalence of progressive renal disease has generally been lower in type 2 diabetes than in type 1 disease (Cowie CC, 1989). However, this observation may not apply to all groups with type 2 diabetes, some of whom have had a more ominous renal prognosis. Studies in type 2 diabetic patients from Western Europe and in Pima Indians from Arizona showed rates of progression to nephropathy similar to those of type 1 diabetic patients. The risk of developing ESRD is much higher in black than in white American patients with type 2 diabetes.

As previously described, however, the use of modern therapies lowers the incidence of ESRD, even in groups at extremely high risk such as the Pima Indians. In a subsequent study, for example, the incidence of diabetic ESRD was noted to have declined significantly from the period 1991-1994 to the period 1999-2002 (32 to 15 cases per 1000 patient-years, respectively).
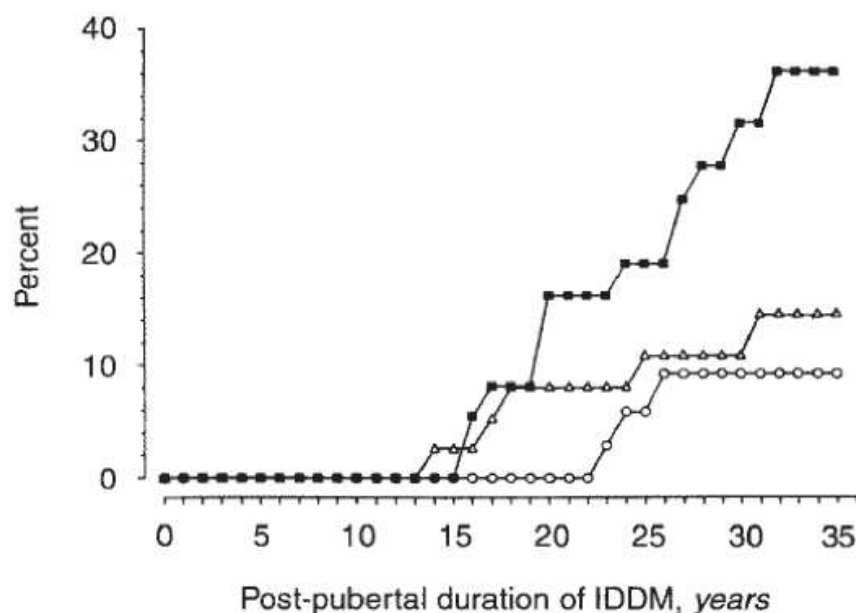

Fig. 2. Cumulative incidence of ESRD according to duration of IDDM and according to tertile of the index of hyperglycemia. Closed rectangles represent the tertile with the highest index of severe hyperglycemia; open triangles represent the middle tertile, and open circles represent the third with the lowest index of hyperglycemia. The differences among the curves are statistically significant, P = 0.017

Data suggest that the renal risk is currently equivalent in the two types of diabetes. Evidence in support of this hypothesis includes the observations in one report that the time to proteinuria from the onset of diabetes and the time to ESRD from the onset of proteinuria were similar in type 1 and type 2 disease (Ritz E, 1999).
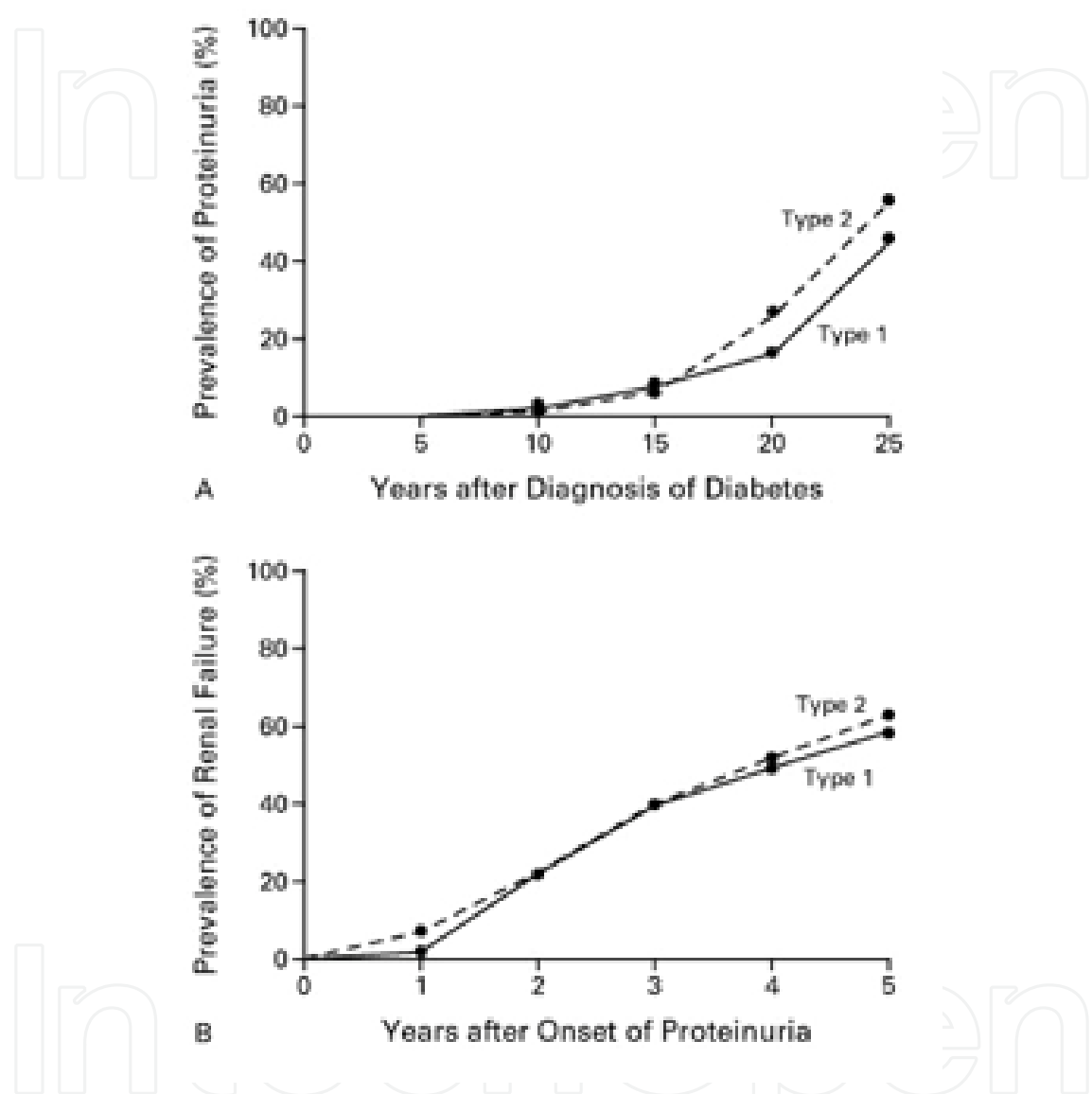

Fig. 3. Cumulative prevalence of persistent proteinuria among patients with type 1 or type 2 diabetes according to the duration of diabetes (Panel A), and cumulative prevalence of renal failure among patients with type 1 or type 2 diabetes according to the duration of proteinuria (Panel B).

Glycemic control, systemic blood pressure levels, and genetic factors seem to be very important in determining diabetic nephropathy risk. Other factors such as lipid levels, smoking habits, and vitamin D intake may also have a role in modulating this risk.
As with type 1 diabetes, some patients with microalbuminuria due to type 2 diabetes, particularly those with good glycemic control, experience regression of microalbuminuria (Araki S, 2005)

3. Natural history and clinical course

The course of renal involvement in type 1 diabetes can be divided in five stages. **Stage I**, present at diagnosis, is that of renal hypertrophy-hyperfunction. At this stage, patients at risk and not at risk of diabetic nephropathy cannot be clearly separated. A 25 to 50 percent elevation in the glomerular filtration rate (GFR) is seen early in the course in up to one-half of patients with type 1 diabetes mellitus, an abnormality that is exaggerated after ingestion of a protein load.
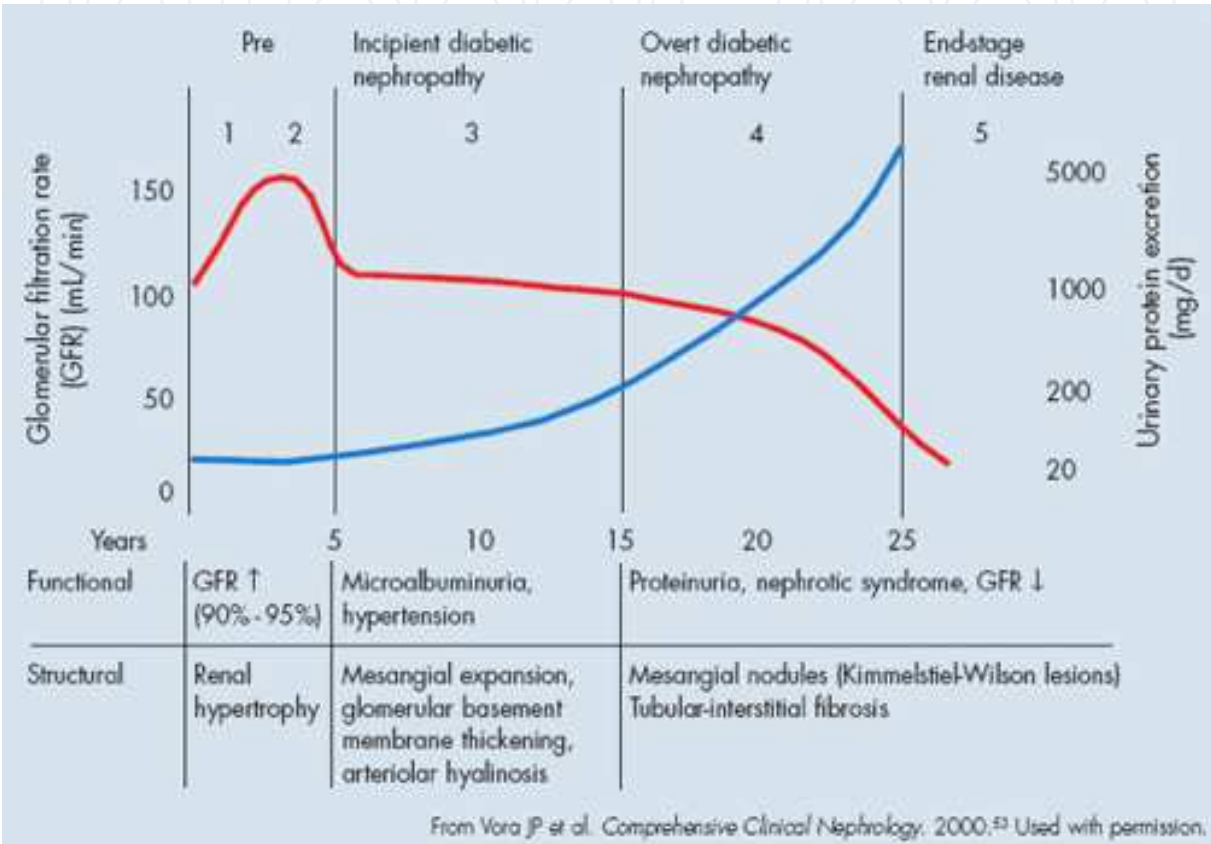

Fig. 4. The five stages of renal involvement in type 1 diabetes

This increase in GFR could be explained by a relative increase in glomerular capillary pressure and/or ultrafiltration coefficient. Glomerular hypertrophy and increased renal size typically accompany the rise in GFR. In an inception cohort study of adult-onset type 1 diabetic subjects, a greater albumin excretion rate within the normal range, male gender, higher mean blood pressure and hemoglobin A1c, and shorter stature were independent predictors of development of microalbuminuria over 18 years of follow-up.

Hyperfiltration also occurs early in the course of type 2 diabetes (Vora JP, 1992). The degree of hyperfiltration and the course of the GFR in type 2 diabetes mellitus was evaluated in more detail in a study of 194 Pima Indians of the Gila River Indian Community in Arizona who have the world's highest incidence of Non-Insulin-Dependent Diabetes Mellitus (NIDDM) (Nelson RG, 1996). The following results were noted:

- In 31 patients with a normal glucose tolerance test, the mean GFR was 123 mL/min
- In 29 patients with impaired glucose tolerance, the mean GFR was 135 mL/min
- In 30 patients with newly diagnosed type 2 disease, the mean GFR was 143 mL/min

- In 70 patients with overt diabetes for more than five years and either normal albumin excretion or microalbuminuria, the mean GFR was 153 mL/min; in 34 similar patients with overt proteinuria, the mean GFR was 124 mL/min
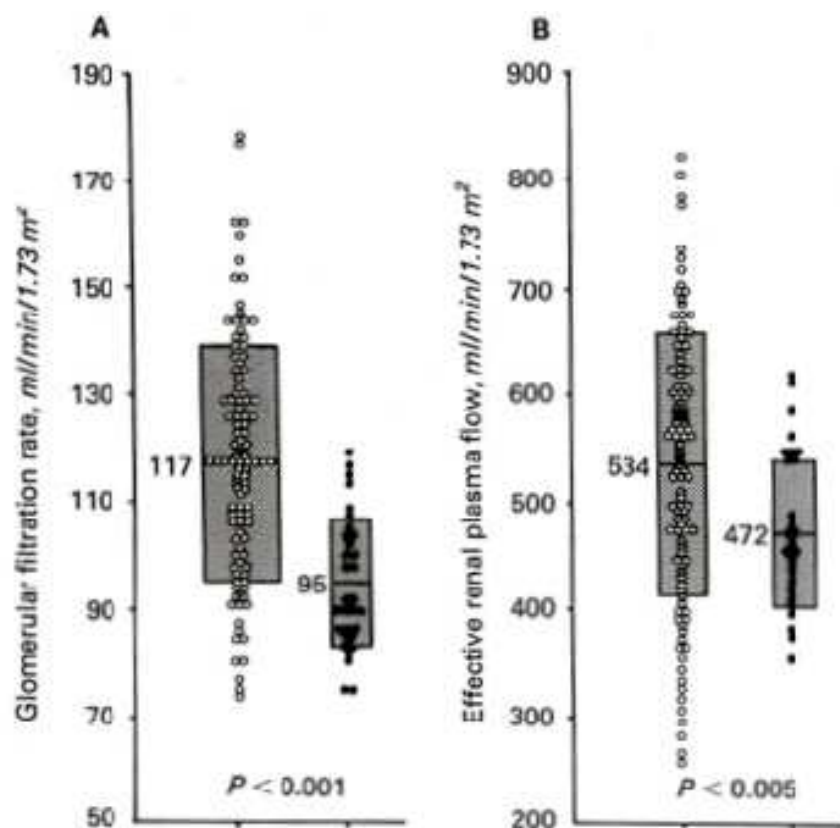

Fig. 5. Glomerular filtration rate and effective renal plasma flow in non-insulin dependent diabetics (NIDDMs) (N= 110) and normal subjects (N = 32).

After four year follow-up, the GFR rose 14 percent in patients with impaired glucose tolerance, 18 percent in newly diagnosed patients, was stable in those with microalbuminuria, and fell 35 percent in those with overt proteinuria. This pattern is consistent with the hypothesis that hyperfiltration causes progressive glomerular damage. However, the base-line glomerular filtration rate in the diabetic subjects predicted neither increasing urinary albumin excretion nor declining glomerular filtration during four years of follow-up, suggesting that hyperfiltration itself is not the principal factor in the development or progression of nephropathy. Higher urinary albumin excretion at base line, however, did predict increasing albuminuria and, in subjects with macroalbuminuria, declines in the GFR; these findings suggest that enhanced protein flux across the glomerular capillary wall contributes to progressive glomerular damage. Proteinuria also predicts the progression of renal disease in patients with nondiabetic renal disease.

Studies in experimental animals indicate that dilatation of the afferent (precapillary) glomerular arteriole plays an important role in the hyperfiltration response, by raising both the intraglomerular pressure and renal blood flow. (Bank N, 1991) A role for hormones is suggested by the ability of a chronic infusion of a somatostatin analogue octreotide to partially reverse both the early hyperfiltration and the increase in renal size in type 1 diabetic patients (Serri O, 1991). There was, however, a fall in the plasma concentration of

insulin-like growth factor I (IGF-1), which is produced in part within the kidney. Although the pathogenetic role of IGF-1 is unproven, it is of interest that infusion of this hormone in normal subjects can replicate the findings seen in diabetics — renal vasodilatation and an elevation in GFR. Similar hemodynamic changes plus renal hypertrophy can be induced by IGF-I in experimental animals.
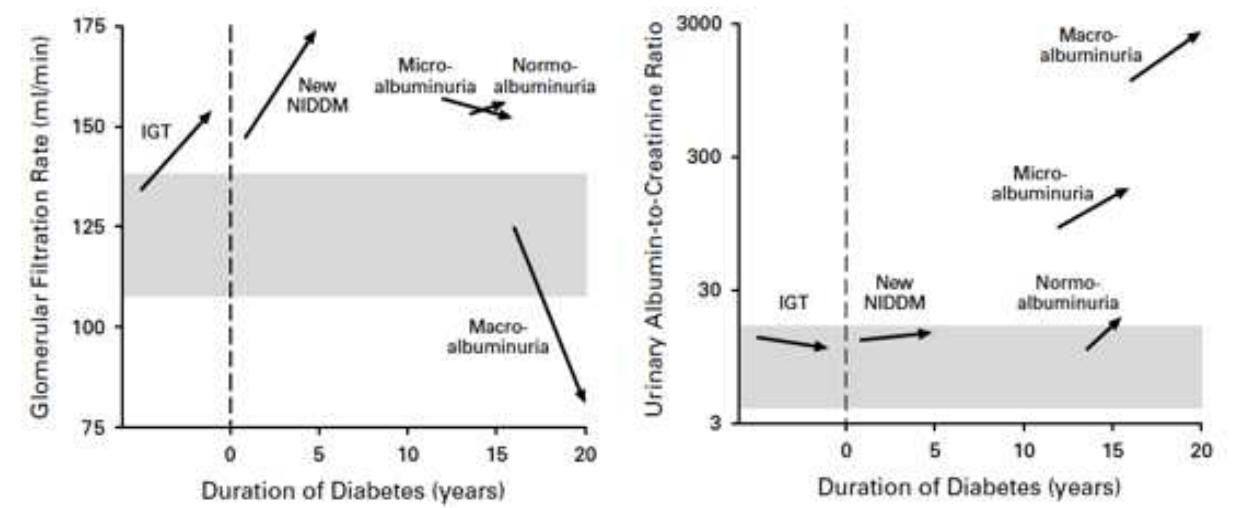

Fig. 6. Changes in the Mean Glomerular Filtration Rate and Median Urinary Albumin-to-Creatinine Ratio from Base Line to the End of Follow-up in Subjects with Impaired Glucose Tolerance (IGT), Newly Diagnosed Non-Insulin-Dependent Diabetes Mellitus
(New NIDDM), NIDDM and Normal Urinary Albumin Excretion (Normoalbuminuria), NIDDM and Microalbuminuria, and NIDDM and Macroalbuminuria.

Each arrow connects the value at the base-line examination and the value at the end of follow-up. The dashed line indicates the time of diagnosis, and the shaded area the 25th through 75th percentiles of values in subjects with normal glucose tolerance. Albumin was measured in milligrams per liter and creatinine in grams per liter.

Several other factors directly related to hyperglycemia also may be important, including the intracellular accumulation of sorbitol and the formation of glycosylated proteins (Passariello N, 1993). The enzyme aldose reductase converts intracellular glucose to sorbitol, which then accumulates within the cells. Studies in hyperfiltering humans with type 1 diabetes have shown that the chronic administration of an aldose reductase inhibitor (tolrestat) lowers the GFR toward normal.

Stage II is defined by the presence of detectable glomerular lesions in patients with normal albumin excretion rates and normal blood pressure levels. Normoalbuminuric patients with more severe glomerular lesions might be at increased risk of progression. Patients can remain in stage 2 for the remainder of their lives. However, those in whom nephropathy is destined to progress further will at this stage exhibit a loss of the normal nocturnal blood pressure decline (i.e., night/day ratios >0.9 and non-dipping) as an early diabetic nephropathy indicator that often precedes the development of persistent microalbuminuria. Microalbuminuria, typically occurring in 2 to 5 percent of patients per year, defines **stage III.** Patients with microalbuminuria are referred to as having incipient nephropathy. Microalbuminuria (a sign of endothelial dysfunction that is not necessarily confined to the kidney) may be present earlier, particularly during adolescence and in patients with poor

glycemic control and high-normal blood pressure levels. Compared with normoalbuminuric patients, patients with persistent microalbuminuria have threefold to fourfold greater risk of progression to proteinuria and ESRD. Current studies indicate that between 20% and 45% of microalbuminuric type 1 diabetic patients will progress to proteinuria after about 10 years of follow-up, whereas 20% to 25% will return to normoalbuminuric levels (Hovind P, 2004) and the rest will remain microalbuminuric. At this stage, glomerular lesions are generally more severe than in the previous stages, and blood pressure tends to be increasing, often into the hypertensive range. Other laboratory abnormalities, such as increased levels of cholesterol, triglycerides, fibrinogen, Von Willebrand's factor, and prorenin, can be detected in some patients. Diabetic retinopathy, lower extremity amputation, coronary heart disease, and stroke are also more frequent in this group.

The normal rate of albumin excretion is less than 20 mg/day (15 µg/min); persistent albumin excretion between 30 and 300 mg/day (20 to 200 µg/min) is called microalbuminuria and, in patients with type 1 diabetes, persistent microalbuminuria may be indicative of early diabetic nephropathy unless there is some coexistent renal disease.

It was initially thought that microalbuminuria precedes the loss of glomerular filtration rate (GFR) in patients with type 1 diabetes. However, some patients with normoalbuminuria or microalbuminuria have significant reductions in GFR prior to the development of macroalbuminuria. Loss of renal function, which was defined as an estimated decrease in GFR of more than 3.3 percent per year, occurred in 9 percent of patients with normoalbuminuria and 16 percent with regression of microalbuminuria. Loss of renal function occurred much more frequently (32 and 68 percent) in patients with stable or progressive microalbuminuria, respectively.

Patients with newly diagnosed type 2 diabetes in which 6.5 percent had microalbuminuria and 0.7 percent had macroalbuminuria at the time of diagnosis. The rate of microalbuminuria at the time of diagnosis of type 2 diabetes may be higher in older patients. There are at least two possible explanations for the presence of microalbuminuria at the time of diagnosis of type 2 diabetes: the patients had previously undiagnosed diabetes or some other disease was responsible for the microalbuminuria. Forty to 50 percent of patients with type 2 diabetes who have microalbuminuria eventually die of cardiovascular disease; this is three times as high a rate of death from cardiac causes as among patients who have diabetes but have no evidence of renal disease.

As with type 1 diabetes, some patients with microalbuminuria and type 2 diabetes regress to normoalbuminuria. At six years, regression occurred in 51 percent, while progression to macroalbuminuria occurred in 28 percent. Several factors (short duration of microalbuminuria, better glycemic and blood pressure control, and the use of ACE inhibitors or angiotensin II receptor blockers) were independently associated with remission.

Stage IV occurs after 10 to 20 years of diabetes and is characterized by the presence of dipstick-positive proteinuria: proteinuria of greater than 300 mg/d. Hypertension is present in about 75% of these patients, and reduced GFR and dyslipidemia are also common. Retinopathy and peripheral and autonomic neuropathy are present in most patients. In addition, the risk for cardiovascular events is extremely high, and asymptomatic myocardial ischemia is frequent. Without therapeutic interventions, GFR declines by about 1.2 mL/min/month in proteinuric type 1 diabetic patients. In type 2 diabetic patients, Once macroalbuminuria is present, creatinine clearance declines at a rate that varies widely from patient to patient; the average reduction is 10 to 12 ml per minute per year in untreated patients. Hypertension and proteinuria may accelerate the decline in the glomerular filtration rate and the progression to end-stage renal disease.

Progression to ESRD (**stage V**) occurs 5 to 15 years after the development of proteinuria. Renal replacement therapy—either dialysis or transplantation is required at this stage.

4. Pathogenesis

Diabetic nephropathy occurs as a result of a complex yet incompletely understood interaction between hemodynamic and metabolic factors (Cooper, M., 2001). Hemodynamic factors that contribute to the development of diabetic nephropathy include increased systemic and intraglomerular pressure, as well as activation of vasoactive humoral pathways including the renin angiotensin system and endothelin (G.M. Hargrove, 2000). These hemodynamic pathways activate intracellular second messengers such as protein kinase C (PKC), Mitogen-activated protein (MAP kinase) (M. Haneda, 1997), nuclear transcription factors such as NF-kB and various growth factors such as the prosclerotic cytokine, TGF-β and the permeability enhancing growth factor, vascular endothelial growth factor, VEGF.

Glucose dependent pathways are also activated within the diabetic kidney and result in enhanced oxidative stress, renal polyol formation (Dunlop ME, 2000) and the accumulation of advanced glycation end products (AGEs). In combination, these pathways ultimately lead to increased renal albumin permeability and extracellular matrix accumulation, resulting in increasing proteinuria, glomerulosclerosis and ultimately tubulointerstitial fibrosis.

5. Hemodynamic pathways

Glomerular hyperperfusion and hyperfiltration are the early signs resulting from decreased resistance in both the afferent and efferent arterioles of the glomerulus. Afferent arteriole seems to have a greater decrease in resistance than the efferent, which in fact may have increased resistance. Many factors have been reported to be involved in this faulty autoregulation, including nitric oxide, prostanoids, vascular endothelial growth factor (VEGF), TGF-β1, and the renin angiotensin system, specifically angiotensin II. These early hemodynamic changes predispose to albumin leakage from the glomerular capillaries and overproduction of mesangial cell matrix, as well as thickening of the glomerular basement membrane and injury to podocytes (Ziyadeh, F 2008). In addition, increased mechanical strain from these hemodynamic changes can induce localized release of certain cytokines and growth factors (Wolf, G.F.N, 2007)
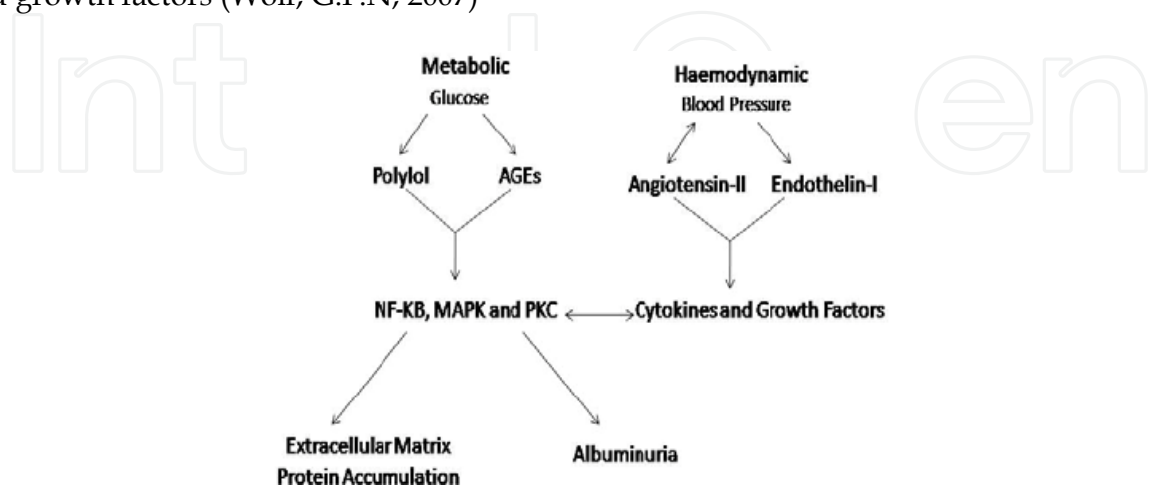

Fig. 7. Interaction of hemodynamic and metabolic pathway, cytokines and intracellular signaling molecules mediating diabetic nephropathy

The action of vasoactive hormones, such as angiotensin II and endothelin are mediator of renal hemodynamic changes. Glomerular hypertension and hyperfiltration contribute to the development of diabetic nephropathy because use of renin–angiotensin blockers preserves kidney function and morphology. Blockade of the renin-angiotensin–aldosterone system antagonizes the profibrotic effects of angiotensin II by reducing its stimulation of TGF-β1 (Hilker, KF, 2005). Support that such profibrotic effects underlie diabetic nephropathy has also been provided by study of an animal model of diabetic nephropathy (Nagai Y, 2005). Transient blockade of the renin–angiotensin system (for 7 weeks) in prediabetic rats reduced proteinuria and improved glomerular structure. Additionally, the administration of an angiotensin converting-enzyme inhibitor to patients with type 1 diabetes and nephropathy appears to reduce serum concentrations of TGF-β1. A correlation exists between decreased levels of TGF-β1 in serum and urine and renal protection, as determined by changes in the glomerular filtration rate.

5.1 Renin-angiotensin system in diabetic nephropathy

The renin-angiotensin system (RAS) has been extensively studied in diabetes. Earlier studies centered on the systemic RAS, and the data obtained have been conflicting, with stimulation, suppression, and no change in the system being reported (Wolf, G, 2007). The factors that influence the systemic RAS in addition to the different stages of disease and species studied may explain many of these divergent findings. However in various diabetic models, increased renal renin content relative to plasma renin levels has generally been found, thus suggesting impaired renal renin release into the circulation. In clinical diabetic nephropathy, there is decreased plasma rennin activity that may be due to nonenzymatic glycation of prorenin with decreased conversion to active renin. Thus, diabetic nephropathy has traditionally been considered a "low renin" state. However, plasma renin activity may not accurately reflect activity of the RAS in the kidney.

Another problem has been the difficulty of accurate measurement of plasma angiotensin-II (Ang II), which is an important issue because discordance can exist between plasma renin and Ang II levels. More recently, the intrarenal RAS has been the focus of extensive study. Abundant evidence indicates the existence of local tissue RASs that are regulated independent of plasma RAS (Ballerma, B.J., 1984). It was reported that glomerular Ang II receptors decrease in the diabetic rat 3 to 4 weeks after induction of the disease. Downregulation of glomerular Ang II receptors implies that intra- renal Ang II generation may be increased. The density of Ang II receptors in the proximal tubules was reported to be reduced in diabetic rats and was accompanied by decreased mRNA expression for the AT1 receptor (Cheng, H.F, 1994). Recently, AT1 receptor density has also been shown to be decreased in mesangial cells when incubated in high-glucose media (Amiri F, G, 1999). ACE activity in whole kidney is low in diabetes.

However, this is probably due primarily to mesangial RAS. Staining for ACE has been found to be enhanced in glomeruli and vasculature of diabetic rats and in patients with diabetic nephropathy (Mizuiri, S., 1998). These data suggest that the term "intrarenal" RAS is oversimplistic, in as much as the vascular RAS (vessels and glomeruli) appears to be regulated differently from the tubulointerstitial RAS. Angiotensin receptor blockers (ARBs) enhance the renal vasodilation in patients with diabetes (despite the presence of low plasma rennin activity), again supporting the concept that the renal vascular RAS is activated in several intrarenal compartments including the glomeruli by several orders of magnitude higher than those found systemically. This shows the existence of both local RAS acting is

independently of the systemic RAS and also is consistent with the finding that in most renal cell culture studies, effects of Ang II are observed at substantially higher concentrations (about 0.01-1.0 mmol/L) than those found in the systemic circulation.

5.2 Vaso-active hormones

Endothelium is an interior covering of blood vessels. There are various biological functions of endothelium and it regulates vascular tone and maintains free flow of blood in vessels (Escandon, J.C, 2001). The luminal surface of every blood vessel, forms a physical and metabolic barrier to circulating elements. The endothelium is an important endocrine organ and releases a number of vasoactive hormones, including endothelin (ET-1) (Ulker, 2003), endothelium-derived hyperpolarizing factor (EDHF: nitric oxide and prostacyclin). Endothelin-1 is a potent vasoconstrictor, Endothelium-derived hyperpolarizing factor is still a controversial subject of vascular biology. Endothelial cells of every blood vessel release nitric oxide and prostacyclin and they form a particular partnership in the regulation of vascular and platelet function.

5.3 Nitric Oxide

Nitric Oxide (NO), originally identified as "endothelial derived relaxing factor," is a ubiquitously utilized signaling molecule that regulates a wide variety of organ and cellular functions, including renal hemodynamics and salt and water regulation. NO is generated enzymatically from the amino acid L-arginine by one of three specific nitric oxide synthases: "neuronal"(NOS1 or nNOS), "endothelial" (NOS3 or eNOS), or "inducible" (NOS2 or iNOS). Many, but not all, of the intracellular signaling pathways activated by NO are mediated by activation of guanylate cyclase, which increases intracellular levels of cyclic guanosine monophosphate.

All three NOS isoforms are present in the mammalian kidney, with both distinct and overlapping patterns of distribution. In normal kidney, NOS1 is highly expressed in the macula densa and glomerular parietal epithelium, as well as in the medulla in the collecting ducts and thin ascending limb. NOS2 is expressed in the endothelium of glomerular capillaries and afferent and efferent arterioles, renal arteries, and descending vasa recta, as well as in proximal tubule and medullary thick ascending limb. NOS3 is also expressed in tubules, including S3 segments of the proximal tubule, medullary thick ascending limb, and collecting duct, in addition to arcuate arteries and vasa recta bundles (Kone, BC, 1997). Both in vivo and in vitro studies have provided conflicting results regarding NOS expression and NO production in diabetes. Most but not all studies in cultured renal cells have determined decreased NO production in response to hyperglycemia (Komers R, 2003).

It has been established for a long time that the principal risk factors that affect the development and progression of diabetic nephropathy includes uncontrolled hyperglycemia, hypertension (systemic and glomerular) and activation of RAAS. All these three factors have been shown to modulate intra renal NO generation either directly or through signaling pathways. The role of NO in affecting the renal structure and function is very complicated and depends on several factors including the stage of diabetic renal disease, isoforms of NOS involved, structures in the kidney, and influence of other factors in diabetic milieu. The complex metabolic milieu in diabetes triggers several pathophysiological mechanisms that simultaneously stimulate and suppress intrarenal NO production. The net effect on renal NO levels depends on the mechanisms that prevail in a given stage of the disease process.

5.3.1 Dual role of Nitric Oxide in diabetic nephropathy

The currently available evidence enables us to reasonably conclude that early diabetic nephropathy is associated with increased renal NO production mediated primarily by constitutively released NO through eNOS or NOS III activation. There is some contribution to this augmented NO production through nNOS (NOS I) derived enhanced synthesis, particularly from macula densa region of the kidney. Together the increased intrarenal NO generation contributes to the development of glomerular hyperfiltration and microalbuminuria that characterize early diabetic nephropathy.

On the other hand, advanced diabetic nephropathy with severe proteinuria, hypertension and renal failure is associated with a state of progressive NO deficiency. As the duration of diabetic state increases, factors that suppress NO bioavailability prevail. Many factors including activation of protein kinase C, activation of TGF-beta, NO quenching by advanced glycosylation end products (AGE) contribute to the NO deficient state – either directly or by inhibiting and/or by post translational modification of activity of NOS isoforms. Other inhibitors of NOS enzyme such as asymmetric dimethylarginine (ADMA) accumulate in diabetic nephropathy and may contribute to progression of DN and such association has also been observed in other microvascular complications such as retinopathy. Most of these changes are mediated by endothelial and partly inducible NOS in the chronic advanced stage of DN. Progressive loss of renal parenchyma also contributes partially to the NO deficiency since kidney is a major source of L-arginine, the sole precursor of NO. These changes and the factors affecting them are discussed well in a review (Prabhakar 2005) and schematically represented in the figure below.
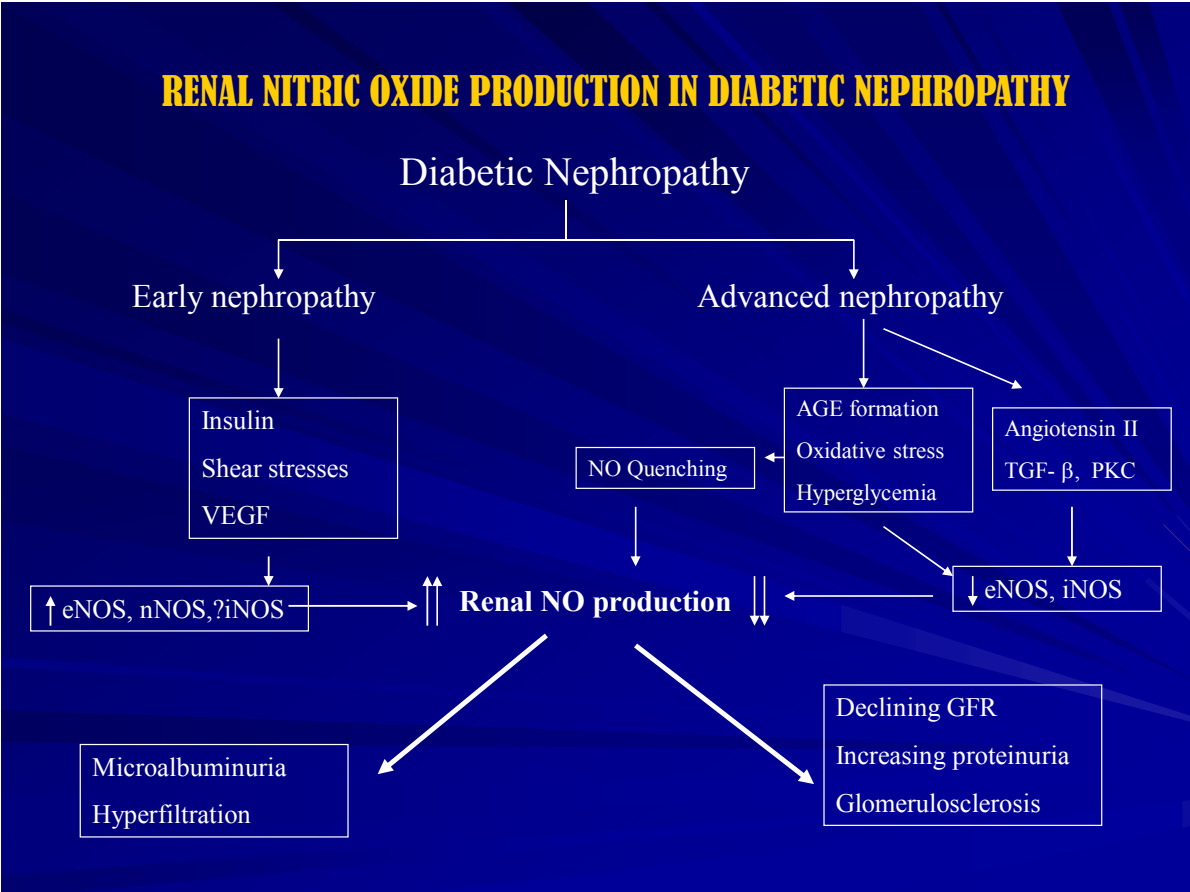

Fig. 8. Role of Nitric Oxide in Diabetic Nephropathy

In a recent study, the natural history of renal manifestations have been described in ZSF_1 rats, a recently developed rodent model of type 2 diabetes who developed obesity and hyperglycemia by 20 weeks of age on a high-carbohydrate diet. They also developed systolic and diastolic hypertension, hypercholesterolemia, profound hypertriglyceridemia, proteinuria, and renal failure. Renal histology demonstrated changes consistent with early diabetic nephropathy, including arteriolar thickening, tubular dilation and atrophy, glomerular basement membrane thickening, and mesangial expansion. Furthermore, renal nitric oxide production was decreased, and homogenates from renal cortices demonstrated reduced expression of renal endothelial and inducible nitric oxide synthases. These changes were associated with increased urinary levels and renal expression of 8-hydroxydeoxyguanosine, an indicator of mitochondrial oxidative stress, as well as with increased renal peroxynitrite formation. Administration of either insulin or the antioxidant alpha-lipoic acid decreased proteinuria and oxidative stress, but only the former slowed progression of renal failure (Prabhakar 2007).

5.4 Prostacyclin

The first step in prostacyclin synthesis is the liberation of arachidonic acid from membrane-bound lipids via the enzymatic actions of phospholipase A2 (PLA2). In endothelial cells, phospholipase A2 activation is a calcium-dependent step. Once liberated, arachidonic acid is available for metabolism by cyclooxygenase (COX). Cyclooxygenase is present in two isoforms: COX-1 and COX-2. Cyclo-oxygenase-1, like NOSI or NOSIII, is constitutively expressed, while COX-2, like NOSII, is induced at sites of inflammation and/or by PAMPs. In healthy endothelial cells, COX-1 is the predominate isoform. Cyclooxygenase has two enzymatic activities: firstly, an oxygenase step forms prostaglandin (PG) G2; and secondly, a peroxidase step, which forms PGH2 from PGG2. Prostaglandin H2 is the substrate for a range of downstream prostaglandin synthase enzymes, including prostacyclin synthetase (PGIS), the actions of which result in the formation of prostacyclin. Endothelial cells are enriched in cyclooxygenase-1(COX-1) and PGIS, which is why, when phospholipase A2 is activated, prostacyclin is the predominant metabolite made. It is important to note that in platelets, which also express predominantly COX-1, thromboxane is the principal product made. This is because platelets express mainly thromboxane synthase with negligible levels of PGIS.

5.5 Endothelin1

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide produced by vascular endothelium from big ET-1 (Xu 1994) via specific cleavage by endothelium converting enzyme (ECE). ET-1 produces its actions by acting on endothelin ETA and ETB receptors (Haynes, 1993). ETA receptor predominates in vascular smooth muscle cells and mediates vasoconstriction in both large and small blood vessels where as ETB receptors on endothelial cells mediate vasodilation through the production of nitric oxide and prostacyclin (Verhaar, MC, 1998). ET-1 is involved in the pathogenesis of cardiovascular disorders such as hypertension and heart failure including diabetic nephropathy (Benigni 1998). Diabetes mellitus induces the renal overexpression of ET-1 in the glomeruli and tubular epithelial cells leading to progression of diabetic nephropathy. It was shown that diabetes-induced elevated level of renal ET-1 might induce glomerular hyperperfusion and damage tubulointerstitium in rats. The diabetes-induced elevated level of renal ET-1 was noted to accelerate the progression of diabetic nephropathy in rats31. It has been documented that ET-1 activates a variety of

signaling systems to induce contraction, hypertrophy, proliferation, and extracellular matrix accumulation in mesangial cells (Sorokin, 2003). The detrimental role of ET-1 in pathogenesis of diabetic nephropathy has been confirmed by the fact that diabetes induced elevated level of renal ET-1 is associated with an expansion of mesangial cells and collagen deposition in the glomeruli of diabetic mice. It has been recently demonstrated that ET-1 mediated activation of ETA receptor induced the renal TGF-β production and inflammation in diabetic rats (Sasser, 2007). Treatment with CPU0213, a dual ETA/ETB receptor antagonist has been found to improve the renal function in rats with diabetic nephropathy by suppressing Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, suggesting that ET-1 contributes to the pathogenesis of diabetic nephropathy via upregulation of NADPH oxidase mediated ROS production in renal cells (Xu, M., 2009). Thus under pathological conditions, prevention of endothelin-mediated various signaling pathways may provide an alternative approach to treat diabetic nephropathy.

5.6 Urotensin

Urotensin II (UII) is an 11-amino acid vasoactive peptide, recently identified as the ligand for a novel G protein-coupled receptor, GPR-14 (renamed urotensin receptor [UT]). In addition to its potentvasoconstrictive actions, UII also has trophic and profibrotic effects, leading to its implication in the pathogenesis of heart failure. However, it has been noted that elevated plasma levels of UII in association with renal impairment and diabetes and diabetic nephropathy. Urotensin-II, an endogenous vasoconstrictor, has been suggested to be involved in the pathogenesis of vascular endothelial dysfunction (VED) (Maguire, J.J., 2002). Urotensin-II increases the activity of NADPH oxidase and plasminogen activator inhibitor-1 (PAI-1) and cause decrease in endothelium dependent relaxation (Watanabe, T., 2006). The overexpression of urotensin-II in endothelial cells cause VED by increasing the expression of type 1 collagen and formation of ROS.

6. Metabolic pathway

Advanced glycosylation end products or AGEs are a chemically heterogeneous group of compounds formed as a result of the ''Maillard reaction'' when reducing sugars react non-enzymatically with amine residues, predominantly lysine and arginine, on proteins, lipids and nucleic acids. While the initial stage of the reaction leading to the formation of reversible glycosylation proteins termed Schiff bases is rapid and glucose dependent, a much slower reaction over a period of days results in the formation of the more stable Amadori product. These early glycosylation products accumulate predominantly on long lived proteins such as vessel wall collagen and crystallines (Brownlee, 2001), undergoing a series of in vivo rearrangements to form irreversible, complex compounds and cross-links, termed AGEs.

Once AGE related cross-links form on proteins, they become resistant to proteolytic degradation (Thomas, 2005). As well as their non-receptor mediated effects; AGES can exert their biological effects through receptor-mediated mechanisms, the most important of which is the receptor for advanced glycation end products (RAGE). RAGE is a signal transduction receptor that belongs to the immunoglobin superfamily and is expressed on a number of cell types including monocytes/macrophages, endothelial cells, renal mesangial cells and podocytes (Yan, S.F., 2004). Binding of AGEs to the RAGE receptor activates a number of pathways implicated in the development of diabetic complications, specifically diabetic

renal disease. These include enhanced cytosolic reactive species formation, stimulation of intracellular molecules such as PKC and NF-kB and the activation and expression of a number of growth factors and cytokines such as TGF-β and VEGF (Wendt, T., 2003). Indeed, strategies to inhibit the formation of AGEs have been shown to ameliorate diabetic nephropathy.

In the initial study by Soulis-Liparota et al. aminoguanidine, an inhibitor of AGE formation, which acts by scavenging intermediates in the advanced glycation pathway attenuated the rise in albuminuria observed in diabetic rodents, while preventing increases in collagen related fluorescence in isolated glomeruli and renal tubules (Soulis-Liparota, T. 1991). Similar results have been obtained with alagebrium, a putative AGE cross-link breaker. In an experimental study, both aminoguanidine and alagebrium attenuated the albuminuria observed in diabetic rodents (Forbes, J.M., 2003). Furthermore, alagebrium was also beneficial when used as part of a delayed intervention protocol, suggesting that it may be useful in both preventing and retarding diabetic nephropathy (Ziayadeh, F.N., 1991).

A subsequent study confirmed and extended these findings. The early (weeks 16–32) and late (weeks 24–32) administration of alagebrium was again shown to reduce albuminuria in a type 1 diabetic rodent model. As a number of previous groups had demonstrated increases in collagen and other extracellular matrix components in experimental diabetic nephropathy, this study also sought to determine the mechanisms surrounding the improvements in microalbuminuria in diabetic rodent kidneys. The compound was shown to reduce diabetes induced increases in the gene expression of TGF-β1, connective tissue growth factor (CTGF) and collagen IV. Early treatment with alagebrium was also associated with significant structural improvement in the kidney including a reduction in the glomerulosclerotic index and tubulointerstitial area, in conjunction with a reduction in AGE peptide fluorescence in serum and the kidney. Furthermore, a reduction in renal accumulation of the specific AGE, carboxymethyllysine (CML) and decreased RAGE immunostaining was also seen, providing further evidence that accumulation of AGEs is implicated in renal extracellular matrix accumulation in diabetes.

In the setting of diabetes mellitus and long-term hyperglycemia, nonenzymatic modification of proteins (or lipids) by glucose, or its metabolic products, results in their stable modification and altered function. This process is thought to underlie a major pathogenic pathway leading to tissue injury in diabetes. A major pathway for AGE formation involves triose phosphate intermediaries derived from metabolism of glucose. Triose phosphates build up as intracellular glucose increases and can nonenzymatically form the early glycosylation product methyglyoxal by spontaneous decomposition (Degenhardt 1998 and Frye 1998). Amine-catalyzed sugar fragmentation reactions then modify protein lysine residues directly, forming N- (epsilon) - (carboxymethyl) lysine (CML), a major product of oxidative modification of glycated proteins. Alternatively, reaction of terminal amino groups (e.g., on lysine) with glucose itself may form from early glycation products (i.e., Amadori products) that rearrange to produce stable moieties that possess distinctive chemical crosslinking and biologic properties, designated AGEs (Cohen 2003 and Vlassara 1981). Other glucosederived Amadori products and fructose are thought to be potential precursors of 3-deoxyglucosone (3-DG) in vivo. Fructose generated by the aldose reductase pathway is converted into fructose-3- phosphate by the action of 3-phosphokinase (3- PK). This leads to the generation of 3-deoxyglucosone, a central precursor in the generation of an array of AGEs, in particular, CML-adducts and others (3-Deoxyglucosone, 1999) 3-DG can further react with proteins to form pyrrolines or pentosidine.

AGEs have been suggested to represent a general marker of oxidative stress and long-term damage to proteins in aging, atherosclerosis, and diabetes (Wendt T.M., 2003). Renal CML-AGE is increased in diabetes. Immunolocalization of CML in skin, lung, heart, kidney, intestine, intervertebral discs, and particularly in arteries provide evidence for age-dependent increases in CML accumulation in distinct locations, and acceleration of this process in diabetes (Schleicher, E.D., 1997). Immunostaining and immunoblots of diabetic human kidneys show increased CML in diabetic glomeruli, especially in the mesangial matrix and capillary walls (Nathan, D.M., 2005).

6.1 Oxidative stress

Generally, large amount of reactive oxygen species are generated with in nephron by metabolic activity that is counter balanced by a large number of antioxidant enzymes and free radical scavenging systems. Peroxidation of cell membrane lipids, oxidation of proteins, renal vasoconstriction and damage to DNA are the negative biological effects of reactive oxygen species. Unfortunately, hyperglycemia tips the balance towards production of reactive oxygen species, most of which seem to be generated in the mitochondria (Nishikawa, T. 2007). The metabolism of glucose through destructive alternate pathways, such as via PKC activation and advanced glycation end-product formation, in the setting of hyperglycemia also seems partly dependent on reactive oxygen species. Oxidative stress specifically induced by hyperglycemia even before diabetes becomes clinically apparent. DNA damage marker induced by reactive oxygen species is higher in patients with severe nephropathy (i.e. proteinuria versus microalbuminuria). Diabetic nephropathy is linked with severe oxidative stress. This pathway may be responsible for the decreased bioavailability of nitric oxide in the kidney (Prabhakar 2007).

6.2 Reactive oxygen species

Diabetic nephropathy is characterized by excessive deposition of extracellular matrix (ECM) in the kidney, leading to glomerular mesangial expansion and tubulointerstitial fibrosis. Clinical studies have demonstrated that high blood glucose is the main cause of initiation and progression of diabetic vascular complications including nephropathy. High reactive oxygen species (ROS) induced by glucose upregulates TGF-β1 and extra cellular matrix protein (ECM) expression in the glomerular mesangial cells. Hyperglycemia induced ROS generation and ROS-activated signal transduction cascade and transcription factors and overexpression of genes and proteins in glomerular mesangial and tubular epithelial cells lead to ECM accumulation in diabetic kidney.

6.3 Nephrin

Podocytes (specialized visceral epithelial cells) are important for the maintenance of the dynamic functional barrier (Mundel 2002). Nephrin, a protein found in these cells, is crucial for maintaining the integrity of the intact filtration barrier. The renal expression of nephrin might be impaired in diabetic nephropathy. Patients with diabetic nephropathy have markedly reduced renal nephrin expression and fewer electron-dense slit diaphragms compared with patients without diabetes and minimal nephropathic changes or controls (Benigni. 2004). Furthermore, nephrin excretion is raised 17-30% in patients with diabetes (with and without albuminuria) compared with that in individuals without diabetes.

Thus, nephrin excretion could be an early finding of podocyte injury, even before the onset of albuminuria (Kobayashi, 2006). Treatment with blockers of the renin-angiotensin-

aldosterone system might help protect nephrin expression. In a study of patients with type 2 diabetes, treatment with an angiotensin-converting-enzyme inhibitor for 2 years maintained nephrin expression at control levels compared with that in untreated patients with diabetes. By contrast, the expression of two other important podocyte and slit diaphragm proteins, podocin and CD2AP, was similar in the three groups. Comparable decreases in renal nephrin expression were reported in other studies of diabetic nephropathy.

6.4 Vitamin D

Vitamin D has a role beyond the regulation of calcium metabolism. There is evidence that the vitamin D system is also involved in regulation of the immune system, cell growth, and differentiation. Vitamin D binds to its nuclear receptor, and later to the vitamin D response element of target genes, to regulate gene transcription. It also interacts with pathways that are germane to the development and progression of diabetic complications, including the renin–angiotensin system (RAS), hypertension, inflammation, and albuminuria. Three recent randomized trials have shown that the vitamin D analogue—19-nor-1-α-dihydroxyvitamin D2 (paricalcitol) can reduce proteinuria in patients with chronic kidney disease, including those with diabetes.
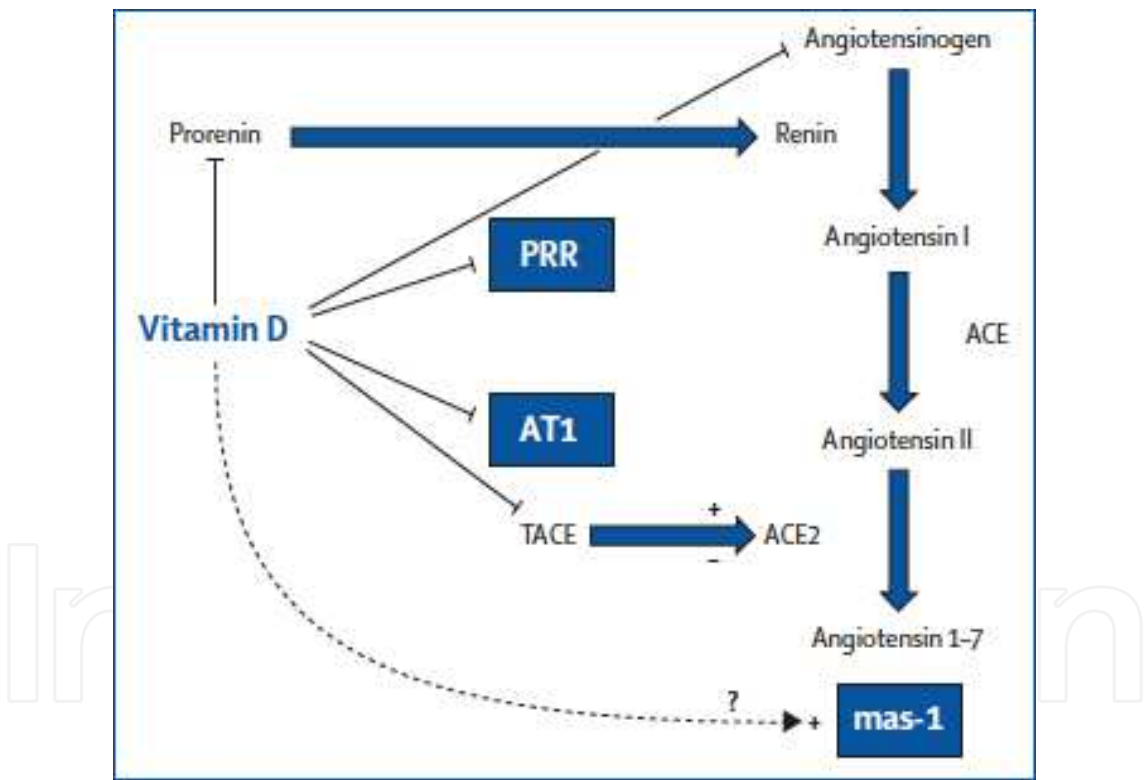
ACE=angiotensin-converting enzyme.
AT1=type 1 angiotensin receptor.
PRR=(pro)renin receptor.
ACE2=angiotensin-converting enzyme 2. TACE=tumor necrosis factor α converting enzyme. mas-1=receptor for angiotensin 1–7.

Fig. 9. Interactions between vitamin D and key elements of the renin–angiotensin system

Vitamin D is a negative regulator of RAS (Li YC, 2002). In experimental chronic kidney disease, paricalcitol reduces the renal expression of renin, the (pro)renin–receptor,

angiotensinogen, and the type 1 angiotensin receptor (Freundlich M, 2008). Vitamin D also inhibits tumor necrosis factor α converting enzyme (TACE) (Dusso A, 2010), which regulates the shedding of angiotensin-converting enzyme 2 (ACE2), itself the major enzyme that metabolizes angiotensin II in the proximal tubule. Diabetes is associated with reduced ACE2 expression; therefore inhibition of TACE expression might improve the balance of RAS in the kidney, and might have additional renoprotective effects by inhibition of the TACE-dependent release of other pathogenic mediators.

Vitamin D has several anti-inflammatory actions, including effects on prostaglandin synthesis, inhibition of nuclear factor κB signaling, and innate immunity, all of which have been implicated in diabetic chronic kidney disease. Vitamin D deficiency is associated with raised concentrations of C-reactive protein. In previous studies, paricalcitol reduced C-reactive protein concentrations in patients with chronic kidney disease, which paralleled the decline in albuminuria

Patients with diabetes have increased rates of vitamin D deficiency (Tanaka H, 2009) especially in those with chronic kidney disease in whom the urinary loss of protein-associated vitamin D magnifies reduced activation of vitamin D by the proximal tubule, and reduced expression of the vitamin D receptor. For these patients, it seems rational to replace vitamin D. Selective analogues that restore vitamin D receptor signaling without risking hypercalcemia or hyperphosphatemia might have particular advantages, because of the aberrant calcification of diabetic vessels. Of note, vitamin D replacement reduced proteinuria in about half of diabetic patients with stage 3 or 4 chronic kidney disease in a placebo-controlled trial (Teng M 2003)

7. Histopathology

The pathogenesis of diabetic nephropathy is complex, and renal pathological lesions are diverse. Most likely, there are many pathogenic pathways, which through composite, interactive routes lead to the histological damage that we see in renal biopsies of patients with diabetic nephropathy (Elisabeth J.J, 2011). Though the number of patients with type 2 diabetes in a worldwide context is increasing and is predicted to be 438 million in 2030, paradoxically, diabetic nephropathy is probably becoming the renal disease per se for which the least renal biopsies are performed. In many centers, clinical parameters in the absence of a renal biopsy will diagnose the patient with diabetic nephropathy. Only if unusual signs or symptoms are present, such as sudden onset nephrotic syndrome, will a renal biopsy be performed, mostly with the aim to exclude other causes than diabetic nephropathy for the patient's clinical presentation. This means that in relation to diabetic nephropathy, co-morbidity is often seen by the pathologist in the renal biopsy, and cases in which diabetic nephropathy alone is present become less frequent. In many recent publications, the diagnosis of diabetic nephropathy was based on clinical symptoms, which, in many studies, also formed the gold standard on the evaluation of intervention therapies meant to prevent, slow down, or even reverse the processes causing diabetic nephropathy.

7.1 Histopathological classification system

Up to 2010, the terminology for histopathological lesions in diabetic nephropathy was variable. The new classification launched in 2010 (Thijs W, 2010) distinguishes four classes, essentially characterized by the absence of histological lesions (class I), mesangial changes (class II), nodular lesions (class III), or a predominance of global glomerulosclerosis (class IV).

Class	Description	Inclusion Criteria
I	Mild or nonspecific LM changes and EM-proven GBM thickening	Biopsy does not meet any of the criteria mentioned below for class II, III, or IV
		GBM > 395 nm in female and >430 nm in male individuals 9 years of age and older[a]
IIa	Mild mesangial expansion	Biopsy does not meet criteria for class III or IV
		Mild mesangial expansion in >25% of the observed mesangium
IIb	Severe mesangial expansion	Biopsy does not meet criteria for class III or IV
		Severe mesangial expansion in >25% of the observed mesangium
III	Nodular sclerosis (Kimmelstiel–Wilson lesion)	Biopsy does not meet criteria for class IV
		At least one convincing Kimmelstiel–Wilson lesion
IV	Advanced diabetic glomerulosclerosis	Global glomerular sclerosis in >50% of glomeruli
		Lesions from classes I through III

LM, light microscopy.
[a]On the basis of direct measurement of GBM width by EM, these individual cutoff levels may be considered indicative when other GBM measurements are used.

Table 1. Histological classification of Diabetic glomerulopathy

7.1.1 Class I: Glomerular basement membrane thickening

The biopsy specimen shows no or only mild, nonspecific changes by light microscopy that do not meet the criteria of classes II through IV. By direct measurements with EM the glomerular basement membrane (GBM) on average is thicker than 430 nm in males 9 years and older and thicker than 395 nm in females. These cutoff levels are based on a deviation from normal GBM thickness plus 2 standard deviations as recently determined. Light microscopic changes in the GBM and epithelial foot process effacement by EM have no influence on the classification.

Class I incorporates cases with certain degree of chronic and other reactive changes (e.g., changes of arterionephrosclerosis, ischemic type changes, or interstitial fibrosis). Diagnosing DN in cases without characteristic light microscopic glomerular lesions may be difficult, especially when a thicker GBM is also seen with aging or hypertension. The presence of arteriolar hyalinosis may be helpful in these cases, although it is not a prerequisite.

GBM thickening is a characteristic early change in type 1 and type 2 DN and increases with duration of disease. GBM thickening is a consequence of extracellular matrix accumulation, with increased deposition of normal extracellular matrix components such as collagen types IV and VI, laminin, and fibronectin. Such accumulations result from increased production of these proteins, their decreased degradation, or a combination of the two. GBM thickening may already be present in type 1 diabetes patients who are normoalbuminuric. GBM thickening has even been described as a "prediabetic" lesion: In patients with proteinuria and isolated GBM thickening but without overt diabetes, 20% were positive on a blood test for diabetes at the time of biopsy, whereas 44% were diagnosed with diabetes at 6 months, and 70% at 2 years after the biopsy was taken. Long-term glucose control and urinary albumin excretion (UAE) correlate strongly with basement membrane thickness.

7.1.2 Class II: Mesangial expansion, mild (IIa) or severe (IIb)

Class II encompasses those patients classified with mild or severe mesangial expansion but not meeting inclusion criteria for class III or IV and is analogous to the previously used term "diffuse diabetic glomerulosclerosis." Mesangial expansion is defined as an increase in extracellular material in the mesangium such that the width of the interspace exceeds two mesangial cell nuclei in at least two glomerular lobules. The difference between mild and severe mesangial expansion is based on whether the expanded mesangial area is smaller or larger than the mean area of a capillary lumen. If severe mesangial expansion is seen in more than 25% of the total mesangium observed throughout the biopsy, the biopsy is classified as IIb. If this is not the case, but at least mild mesangial expansion is seen in more than 25% of the total mesangium, the biopsy is classified as IIa.

Expansion of cellular and matrix components in the mesangium is a hallmark of type 1 and type 2 DN. It can be detected in some patients within a few years after the onset of type 1 diabetes. When the mesangium expands, it restricts and distorts glomerular capillaries and diminishes the capillary filtration surface.

Various indices have been proposed to describe the amount of mesangial expansion in DN. Mauer et al. define mesangial expansion by mesangial fractional volume or volume density (Vv), defined as the fraction or percentage of the cross-sectional area of the glomerular tuft made up by mesangium, expressed in the formula: Vv (mes/glom). Using this formula, many correlations have been made between mesangial expansion and clinical parameters of DN, particularly showing highly inverse correlations exist between Vv (mes/glom) and GFR. There is also a relationship between Vv (mes/glom) and UAE and blood pressure.

Another index to express mesangial expansion is the so-called "index of mesangial expansion" (IME) for DN. The IME is determined by a semiquantitative estimate of the width of mesangial zones in each glomerulus: grade 0 is normal, 1 is twice normal thickness, 2 is three times normal thickness, and so forth; half grades can also be assigned. The mean of the grades for each glomerulus for IME can thus be determined from a single biopsy. The IME closely correlates with the Vv (mes/glom).

In other classifications, mesangial expansion is defined in more practical ways, such as in the new classification for IgA nephropathy in which it is defined as an increase in the extracellular material in the mesangium such that the width of the interspace exceeds two mesangial cell nuclei in at least two glomerular lobules.

7.1.3 Class III: Nodular sclerosis (Kimmelstiel–Wilson lesions)

If at least one convincing Kimmelstiel–Wilson lesion is found and the biopsy specimen does not have more than 50% global glomerulosclerosis it is classified as class III. Kimmelstiel–Wilson lesions appear in type 1 and type 2 diabetes (only one-third of microalbuminuric type 2 diabetic patients had them) as focal, lobular, round to oval mesangial lesions with an acellular, hyaline/matrix core, rounded peripherally by sparse, crescent-shaped mesangial nuclei. Compared to type 1 diabetes, type 2 diabetes

Paul Kimmelstiel and Clifford Wilson first described nodular lesions in glomeruli from eight maturity-onset diabetes patients in 1936. According to Cameron, they barely noted the association with diabetes, and it was Arthur Allen who clarified the association in 105 patients with diabetes in 1941. Nodular sclerotic lesions may also occur in the absence of DN that are clinically related to hypertension, smoking, hypercholesterolemia, and extrarenal vascular disease.
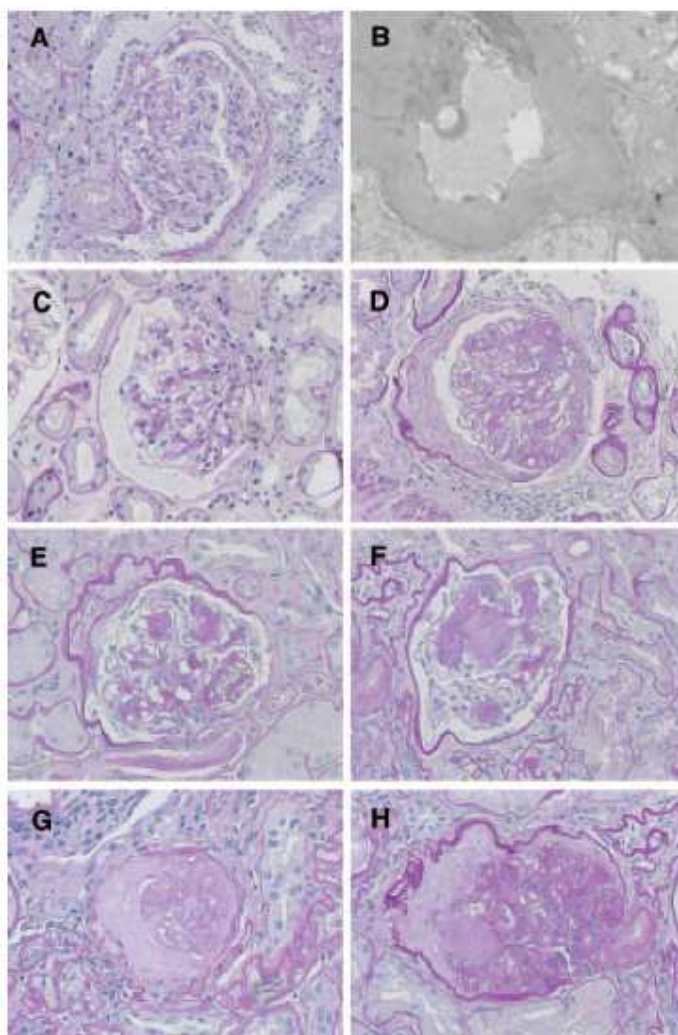
Fig. 10. Representative examples of the morphologic lesions in DN:

(A) Glomerulus showing only mild ischemic changes, with splitting of Bowman's capsule. No clear mesangial alteration.

(B) EM of this glomerulus: the mean width of the GBM was 671 nm (mean taken over 55 random measurements). EM provides the evidence for classifying the biopsy with only mild light microscopic changes into class I.

(C, D) Class II glomeruli with mild and moderate mesangial expansion, respectively. In panel C, the mesangial expansion does not exceed the mean area of a capillary lumen (IIa), whereas in panel D it does (IIb).

(E, F) In panel F is a class III Kimmelstiel–Wilson lesion. The lesion in panel E is not a convincing Kimmelstiel–Wilson lesion, therefore (on the basis of the findings in this glomerulus) the finding is consistent with class IIb. For the purpose of the classification, at least one convincing Kimmelstiel–Wilson (as in panel F) needs to be present.

In panel H, signs of class IV DN consist of hyalinosis of the glomerular vascular pole and a remnant of a Kimmelstiel–Wilson lesion on the opposite site of the pole.

Panel G is an example of glomerulosclerosis that does not reveal its cause (glomerulus from the same biopsy as panel H).

For the purpose of the classification, signs of DN should be histopathologically or clinically present to classify a biopsy with global glomerulosclerosis in **>** 50% of glomeruli as class IV

It is claimed that in the initial stage of developing nodular sclerotic lesions in DN, two important processes take place: lytic changes in the mesangial area called mesangiolysis and detachment of endothelial cells from the GBM. Exactly how these two processes relate remains uncertain. Paueksakon et al. detected fragmented red blood cells in Kimmelstiel–Wilson lesions, which supports the theory that microvascular injury contributes to the pathogenesis of these lesions. Dissociation of endothelial cells may disrupt the connections between the mesangial area and the GBM. This process precedes expansion of the Kimmelstiel–Wilson lesion. These lesions consist of an accumulation of mesangial matrix with collagen fibrils, small lipid particles, and cellular debris.

A completely developed Kimmelstiel–Wilson lesion destroys the normal structure of glomerular tuft with a decrease in mesangial cells, especially in the central area. In 1992, a graphic method of analysis of the position of Kimmelstiel–Wilson lesions demonstrated the nodules were distributed in a horseshoe-shaped area corresponding to the peripheral or intralobular mesangium, excluding the possibility of hyperfiltration as being their main cause of development.

The presence of at least one Kimmelstiel–Wilson lesion associates with longer duration of diabetes and less favorable clinical parameters. In a study of 36 patients with type 2 diabetes, patients with Kimmelstiel–Wilson lesions had more severe overall retinopathy and higher serum creatinine concentrations than those with mesangial lesions alone. In a study of 124 Chinese patients with type 2 diabetes, patients with at least one Kimmelstiel–Wilson lesion had relatively long duration of diabetes mellitus, a poor prognosis, and frequent evidence of diabetic retinopathy. Kimmelstiel–Wilson lesions are often found in combination with mesangial expansion. The occurrence of Kimmelstiel– Wilson lesions is widely considered transitional from an early or moderately advanced stage to a progressively more advanced stage of disease.

7.1.4 Class IV: Advanced diabetic glomerulosclerosis

Class IV implies advanced DN with more than 50% global glomerulosclerosis in which there is clinical or pathologic evidence that the sclerosis is attributable to DN. Glomerulosclerosis in DN is the end point of multifactorial mechanisms that lead to excessive accumulation of extracellular matrix proteins such as collagen types I, III, and IV and fibronectin in the mesangial space, which through stages of mesangial expansion and development of Kimmelstiel–Wilson lesions finally result in glomerulosclerosis. The clustering of sclerotic lesions in columns perpendicular to the kidney surface suggests that vascular factors relating to the interlobular arteries also contribute.

8. Tubulointerstitial lesions, vascular lesions and nondiabetic glomerular lesions

8.1 Tubular lesions

Concomitant tubular basement membrane thickening of nonatrophic tubules is apparent from the development of class II glomerular diabetic lesions and becomes more conspicuous in class III and IV, which is best seen in PAS or silver stains. Interstitial fibrosis and tubular atrophy (IFTA) follow glomerular changes in type 1 DN that ultimately lead to ESRD. A score of 0 is assigned when the biopsy specimen shows no IFTA, a score of 1 is assigned when less than 25% IFTA is present, a score of 2 is assigned when at least 25% but less than

50% of the biopsy has IFTA, and finally, a score of 3 is assigned when at least 50% IFTA is present, which is similar to the scoring in the recently published classification of IgA nephropathy.

Presence of mononuclear cells in the interstitium is a widely recognized finding in DN. Inflammatory interstitial infiltrates comprise T lymphocytes and macrophages. A score of 0 is assigned if interstitial infiltrates are absent, 1 if they only occur around atrophic tubules, and 2 if the inflammatory infiltrate is also in other areas than around atrophic tubules.

8.2 Vascular lesions

According to Stout et al., hyalinosis of the efferent arteriole is relatively specific for DN, but hyalinosis of the afferent arteriole occurs in numerous other settings. Chronic cyclosporine nephropathy is a typical example in which arteriolar hyalinosis occurs outside DN. Tracy et al. also report the presence of arteriolar hyalinosis in kidneys of young patients with coronary heart disease. Efferent arteriolar hyalinosis is an important lesion by which DN is distinguished from hypertensive nephropathy. However, most studies relate arteriolar hyalinosis to clinical parameters, not distinguishing between efferent and afferent arterioles, showing clear correlations with UAE and disease progression.

In addition to characteristic arteriolar hyalinosis, relatively nonspecific arteriosclerosis may be present in the biopsy specimen. Bohle and colleagues found increases in vascular disease associate with more severe glomerular disease. Osterby et al. use a so-called "matrix to media ratio" to investigate the role of arteriosclerosis and find this ratio is increased in patients with microalbuminuria, suggesting that arteriolar matrix accumulation occurs early in the course of DN. By assessing the most severely affected artery in the biopsy and assign a score of 0 if no intimal thickening is present, 1 if intimal thickening is less than the thickness of the media, and 2 if intimal thickening is more than the thickness of the media. Isolated or significant medial thickness may be associated with concurrent hypertension.

9. Other glomerular lesions

In 1994, Stout et al. defined "insudative lesions" as consisting of intramural accumulations of presumably imbibed plasma proteins and lipids within renal arterioles, glomerular capillaries, Bowman's capsule, or proximal convoluted tubules. Insudative lesions in Bowman's capsule are called capsular drop lesions, and in afferent and efferent arterioles they are called hyalinized afferent and efferent arterioles. In glomerular capillaries they are called fibrin cap lesions, although this term is considered obsolete and moreover is amisnomer because the lesion does not contain fibrin; we prefer the term hyalinosis for these lesions.

Capsular drops are mainly located between the parietal epithelium and Bowman's capsule of the glomerulus. Capsular drops are prevalent in advanced DN and associate with disease progression. The common belief, reviewed by Alsaad et al., is that capsular drops are specific but not entirely pathognomonic of DN. Stout et al. report a prevalence of capsular drops in 5.3% of biopsies without diabetes. However, finding a capsular drop in a biopsy can help distinguish DN from other causes of glomerulosclerosis.

By light microscopy, glomerular hyalinosis describes the same staining characteristics as the capsular drop lesion but it occupies the capillary lumen instead of being attached to Bowman's capsule. This lesion is not a specific finding in DN, because similar lesions are recognized in focal glomerulosclerosis, arterionephrosclerosis, and lupus nephritis.

Lesion	Criteria	Score
Interstitial lesions		
IFTA	No IFTA	0
	<25%	1
	25% to 50%	2
	>50%	3
interstitial	Absent	0
inflammation	Infiltration only in relation to IFTA	1
	Infiltration in areas without IFTA	2
Vascular lesions		
arteriolar hyalinosis	Absent	0
	At least one area of arteriolar hyalinosis	1
	More than one area of arteriolar hyalinosis	2
presence of large vessels	–	Yes/no
arteriosclerosis (score worst artery)	No intimal thickening	0
	Intimal thickening less than thickness of media	1
	Intimal thickening greater than thickness of media	2

Table 2. Interstitial and vascular lesions of DN

Finally, there is increasing recognition of abnormalities in the glomerulotubular junctions with focal adhesions called "tip lesions" and atrophic tubules with no observable glomerular opening (so-called "atubular glomeruli"). These lesions are typically found in more advanced stages of nephropathy associated with overt proteinuria.

One of the important questions that need to be validated is whether the new classification system, which makes no distinction between patients with type 1 and type 2 diabetes, is helpful for clinicians. Type 2 diabetes has more heterogenous clinical course with more heterogenous lesions. Type 2 diabetes also has different response towards treatments, and different relationship between diabetic nephropathy and retinopathy. In a study that published by Osterby R, Gall MA, Schmitz A, et al in 1993, virtually all patients with type 1 diabetes with overt nephropathy have retinopathy, whereas less than 50% of patients with type 2 diabetes and diabetic nephropathy have diabetic retinopathy. These figures may have been altered somewhat in the course of time. In 2010, Pedro et al. studied the prevalence and relationship between diabetic nephropathy and retinopathy in a population-based transversal study in northeastern Spain including 8187 type 2 and 488 type 1 diabetes patients. They distinguished between patients with microalbuminuria and those with overt nephropathy. The relationship between microalbuminuria and diabetic retinopathy was different between the types of diabetes, but the relationship between overt nephropathy and diabetic retinopathy was similar in both types. Overt nephropathy, in this study, was a risk factor for diabetic retinopathy in both types.

10. Biomarker studies

A number of studies reported on biomarkers in either plasma or urine for diabetic nephropathy, particularly in relation to type 2 diabetes. A group from Denmark reported on transthyretin, apolipoprotein A1, apolipoprotein C1, and cystatin C as promising biomarkers for diabetic nephropathy in the plasma of patients with type 1 diabetes (Overgaard AJ 2010). Proteomic analysis of plasma from a cross-sectional cohort of 123 type 1 diabetic patients previously diagnosed as normoalbuminuric microalbuminuric, or

macroalbuminuric, gave rise to 290 peaks clusters. Independent component analysis identified 16 candidate peaks that contributed significantly in their respective components with high stability and ability to separate the groups. Four of the peaks were identified as transthyretin, apolipoprotein A1, apolipoprotein C1, and cystatin.

Soluble CD40 ligand derived from platelets and mediating atherothrombosis, was shown to be elevated in type 1 diabetes patients with diabetic nephropathy in comparison with controls. The study was a prospective, observational follow-up study of 443 type 1 diabetes patients with diabetic nephropathy and a control group of 421 patients with longstanding type 1 diabetes and persistent normoalbuminuria. High levels of sCD40L did not predict development of end-stage renal disease (P = 0.85) nor rate of decline in glomerular filtration rate (GFR) (Lajer M 2010).

There were several studies on biomarkers in type 2 diabetes giving rise to a number of new markers. A group from China reported an independent association between plasma levels of osteopontin and the presence and severity of diabetic nephropathy in type 2 diabetes (Yan X 2010). In another study on type 2 diabetes, plasma levels of methylglyoxal, a side-product of many metabolic pathways, was found to be higher in patients with diabetic nephropathy than in those without and, furthermore, were shown to correlate with the urinary albumin: creatinine ratio (Lu J 2011). Fibroblast growth factor 23 (FGF-23), previously reported as a marker for outcome in chronic kidney disease in general, was found to be predictive of renal outcome in type 2 diabetes patients with macroalbuminuric nephropathy (Alkhalaf A, 2010). At baseline, serum FGF- 23 showed a significant association with serum creatinine and proteinuria. FGF-23 was an independent predictor of the primary outcome in this study, defined as death, doubling of serum creatinine, and/or dialysis need. The moderate consistency observed between biomarkers reported in different studies is puzzling, which is probably due to different technologies used and the lack of statistical power in some studies resulting in the reporting of artifacts. Also the distinction between those patients with and without diabetic nephropathy in most of these studies is not proven by biopsy.

It is evident that a huge amount of effort is being put into how to identify diabetic nephropathy in patients with diabetes without being invasive, that is, without taking a renal biopsy, both in clinical practice and clinical research studies. A drawback in most of these biomarker studies pursuing this aim is that no renal biopsy was taken to determine the presence and severity of diabetic nephropathy in the diabetic patients in the first place. Virtually all studies rely on clinical parameters, mostly expressed as the amount of albuminuria, for the diagnosis of diabetic nephropathy. How reliable is this? Chronic renal insufficiency and/or proteinuria especially in type 2 diabetes may stem from chronic renal disease other than classic diabetic nephropathy. In a recent retrospective study of 69 patients with type 2 diabetes with renal biopsies, 52% had non diabetes-related nephropathy (Mou S, 2010). Selection bias most likely plays a role in these data, as renal biopsies are typically performed in these patients if co-morbidity is suspected. It remains, however, most likely that some of the patients included in the biomarker studies were unrightfully given the diagnosis of diabetic nephropathy, and it is uncertain how important this contamination of the data is for the study outcomes. Only one marker study in 2010 was found that did incorporate renal biopsy findings and interestingly, in this study, a classification model was able to reliably differentiate diabetic nephropathy from nondiabetic chronic kidney disease (Papale M 2010). Among the best predictors of this classification model were ubiquitin and b 2-microglobulin.

There are probably also a considerable proportion of patients without albuminuria who are at the beginning stages of diabetic nephropathy. These patients are unrightfully diagnosed as not having diabetic nephropathy. Interesting in this light was the 2010 study by Nielsen et al., which evaluated the new marker of tubulointerstitial damage kidney injury molecule 1 (KIM-1) in type 1 diabetes patients with either normoalbuminuria, microalbuminuria, or macroalbuminuria in comparison with normal controls. Urine KIM1 was elevated in all type 1 diabetes patients in comparison with the normal controls and irrespective of the presence of albuminuria. Thus, normoalbuminuric patients with type 1 diabetes also had elevated urine KIM1. It was therefore concluded that tubular damage may be present at an early stage in all patients, the so-called 'tubular phase' of diabetic nephropathy. Whether or not this means that in fact all patients with type 1 diabetes have a latent form of diabetic nephropathy remained undiscussed, but that would be an interesting hypothesis. Another study that investigated urinary changes in non-overt diabetic nephropathy in type 2 diabetes came from Japan (Araki S,2010). This study included 254 patients with type 2 diabetes of whom 185 were normoalbuminuric and 69 had microalbuminuria. At baseline, urinary type IV collagen levels. were higher in patients with microalbuminuria. During a follow-up study with a median duration of 8 years, the level of urinary type IV collagen inversely correlated with the annual decline in estimated GFR, whereas overt proteinuria did not appear in a majority of patients. Two studies from Malaysia gave similar results, with type IV collagen levels correlating with the amount of urinary albumin in 30 type 2 diabetes patients at baseline Sthaneshwar P, 2010), but also with subsequent GFR change (Katavetin P 2010). Also in New Zealand, urinary collagen IV was studied in diabetes: spot urine samples from 457 unselected patients attending a hospital diabetes clinic were analyzed for albumin, creatinine, and a number of biomarkers including collagen IV (Cawood TJ, 2010). The proportion of patients with abnormal collagen IV increased from 26, 58, and 65%, respectively, across the normoalbuminuria, microalbuminuria, and macroalbuminuria groups. The authors conclude that longitudinal studies are now required to assess whether these biomarkers can detect early renal disease with greater specificity and sensitivity than the albumin: creatinine ratio. Including histopathological findings in these types of studies would certainly make a great contribution to our better understanding of the mechanism leading to diabetic nephropathy.

11. Treatment

Interventions that have been found useful in preventing or retarding the progression of DN include

- Strict glycemic control
- Strict blood pressure control
- Cessation of smoking
- Control of hyperlipidemia and
- Restriction of protein intake.

12. Strict glycemic control

Hyperglycemia is an important risk factor for development of microalbuminuria, progression of established microalbuminuria to macroalbuminuria and impaired glomerular filtration rate (GFR). Additional risk factors for microalbuminuria include older age, male

sex, long duration of diabetes, smoking, obesity, elevated blood pressure, and genetic predisposition. (de Boer IH 2011)

Studies have shown that strict glycemic control delays the development of microalbuminuria, stabilizes or reduces protein excretion in patients with microalbuminuria and overt proteinuria, and slows the rate of progression to chronic renal failure. The Diabetes Control and Complications Trial (DCCT) compared conventional with intensive glycemic management in 1,441 type-I diabetic patients. This study proved that intensive treatment reduced the risks of retinopathy, nephropathy, and neuropathy by 35% to 90% compared with conventional treatment. The absolute risks of retinopathy and nephropathy were proportional to the mean glycosylated hemoglobin (HbA1c) level over the follow-up period preceding each event. Intensive treatment was most effective when begun early, before complications were detectable. These risk reductions were achieved at a median HbA1c level difference of 9.1% for conventional treatment versus 7.3% for intensive treatment. ((The DCCT/EDIC Research Group 2000, The DCCT/EDIC Research Group 2002)) In the combined cohorts, intensive treatment reduced the development of microalbuminuria and clinical albuminuria by 39% and 56%, respectively. The benefits of intensive therapy were also long-lasting and persisted beyond the period of shortest intervention. (The DCCT/EDIC Research Group 2000, The DCCT/EDIC Research Group 2002) Thus, intensive treatment should be started as soon as possible safely after the onset of type 1 diabetes mellitus and maintained thereafter, aiming for a practicable target HbA1c level of 7.0% or less. (Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group 2002). These findings suggest that hyperglycemia has long-term chronic effects on the underlying pathophysiology of microvascular complications. It takes time for improvements in control to negate the long lasting effects of prior prolonged hyperglycemia, and once the biological effects of prolonged improved control are manifest, the benefits are long lasting. However, using the current intensive treatment regimen led to a 3-fold increase in severe hypoglycemic events and to excess weight gain in the DCCT. (DCCT/EDIC research group 2002)

Efforts need to be made to eliminate preventable severe hypoglycemic episodes that result from unsafe patient behavior and decisions, and to avoid inordinate weight gain. Irregular food intake, failure to check blood glucose before planned or unplanned vigorous exercise or before operating a motor vehicle, and excess alcohol ingestion have been identified as risk factors for hypoglycemia and serious complications and must be scrupulously avoided. Mealtime bolus doses of rapid acting insulin must be based on the pre-injection blood glucose level, the anticipated amount of carbohydrate intake and upcoming exercise. Thorough diabetes education and its regular reinforcement should be provided by diabetes nurse and dietitian educators. These professionals can negotiate individualized care plans with patients, give them training in self-management, and provide stimulation, motivation, and positive reinforcement for good self-care behavior, such as frequent self blood glucose monitoring and regular eating habits. While these measures can interfere with patients' lifestyles, they are the current price that must be paid to delay or reduce the risk of microvascular complications. (DCCT/EDIC research group 2002)

13. Blood pressure control

Long term and aggressive antihypertensive treatment induces a progressive reduction in the rate of decline in kidney function. Thus, this modality of treatment can postpone renal

insufficiency in patients with DN. Both systolic and diastolic hypertension markedly accelerate the progression of DN, and aggressive antihypertensive management has been shown to decrease the rate of fall of glomerular filtration rate, increase the median life expectancy, and reduce the need for dialysis and transplantation.

The primary goal of therapy for non-pregnant patients with DM older than 18 years is to achieve a blood pressure less than 130/80 mmHg for patients with proteinuria <1 g/day, and less than 125/75 mmHg for patients with >1 g/day of proteinuria. Angiotensin converting enzyme (ACE) inhibitors and ARBs are recommended as first-line antihypertensive therapy for patients with type-I and type-2 DM. Other agents that can be used include Beta blockers, calcium channel blockers, and diuretics. (American Diabetes Association 2003, Ayodele OE 2004).

Persistent clinical proteinuria is closely associated with the presence of hypertension in IDDM patients. In NIDDM patients the risk of developing clinical proteinuria is increased more than twofold in patients with blood pressure > 165/95 mm Hg compared to those with lower blood pressure after adjusting for age, sex and duration of diabetes. Moreover, in both insulin-dependent and non-insulin-dependent diabetes, the age adjusted total mortality rate is greatly increased in those patients with proteinuria or hypertension (Earle K 1994). Prospective studies have shown that normoalbuminuric patients who progress to microalbuminuria have higher blood pressures (albeit within the normal range) than those who persistently remain normoalbuminuric. Parents of insulin-dependent diabetic patients with nephropathy have a higher prevalence of hypertension and cardiovascular disease compared to patients without nephropathy (Earle K 1994).

ACE Inhibition versus Angiotensin II (Ang II) receptor type 1 blockade in the Renin-Angiotensin System (RAS). RAS is one of the most important physiological regulators of renal function. ACE inhibitors selectively dilate efferent arterioles. This decreases the arterial pressure and in turn reduces glomerular capillary pressure. In addition, Ang II causes mesangial cell growth and matrix production. Numerous animal studies and clinical trials have shown that ACE inhibitors significantly reduce the loss of kidney function in DN. They prevent progression of microalbuminuria to overt proteinuria and several studies evaluated the effect of ACE inhibitor on development and progression of DN.

The landmark study by Lewis et al. [1993], examined the effect of captopril on the progression of DN in patients with type 1 DM (Lewis, EJ 1993). This was measured as the rate of decline in creatinine clearance and the combined end points of dialysis, transplantation, and death. Treatment with captopril was associated with 48% risk reduction for doubling the serum creatinine as compared to placebo. The results of this study were subsequently confirmed by North American Microalbuminuria Study Group and EUCLID study group (EUCLID Study Group 1997, Laffel LM 1995). They extended the observation by showing a protective effect of ACE inhibitors in patients with a variety of renal diseases, including glomerulopathies, interstitial nephritis, nephrosclerosis, and DN. The exception was polycystic kidney disease. Importantly, the protective effect of ACE inhibition was independent of the severity of renal insufficiency. (Lewis, EJ 1993, Brown N 1998)

The MICRO-HOPE (Heart Outcomes Prevention Evaluation) studied the benefit of ramipril in type 2 diabetics. The diabetes sub-study of the Heart Outcomes Prevention Evaluation study showed ramipril reduced the risk of overt nephropathy by 24%. Moreover, ramipril reduced urinary albumin excretion at 1 year and at the end of the study. Thus, ACE inhibitors have also been shown to be renoprotective in patients with type 2 DM. (Heart Outcomes Prevention Evaluation Study Investigators 2000)

Two studies, Irbesartan in Patients with Diabetes and Microalbuminuria (IRMA-2) and Diabetics Exposed to Telmisartan and Enalapril (DETAIL) study examined the effect of ARB's in diabetic patients with microalbuminuria, but without overt DN (Parving HH 2001, Barnett A 2006). It is well known that, in patients with type 2 DM presence of microalbuminuria increases the risk of developing DN 10 to 20 times. IRMA-2 study showed that irbesartan significantly reduced the rate of progression of microalbuminuria to overt DN in patients with type 2 diabetes. Furthermore, the study discovered that irbesartan was associated with significantly more common restoration of normoalbuminuria as compared to standard therapy. All these effects were achieved independent of the systemic blood pressure. The more recent DETAIL study compared the renoprotective effects of ACE inhibitor enalapril and ARB telmisartan. In this head-to-head comparison the authors showed that both treatments were equally effective in preventing the progression of renal dysfunction, measured as decline in the GFR. Reduction of Endpoints in NIDDM with Angiotensin II Antagonist Losartan (RENAAL) study (Brenner BM 2001) showed that treatment with losartan was associated with a 25% decrease in the risk for doubling serum creatinine level. The risk of developing ESRD was also reduced by 28%.

Other individual factors may better define the impact of ACE inhibitors on the progression of renal insufficiency. In particular, Rigat et al described an insertion (I) and deletion (D) polymorphism in the ACE gene that correlates with ACE activity. ACE levels are highest in patients who are homozygous for the ACE D allele and lowest in patients homozygous for the ACE I allele (Rigat B 1990). They are intermediate in those who are heterozygous. Yoshida et al (Yoshida H 1995) later reported a greater reduction in proteinuria in response to ACE inhibition in patients with IgA nephropathy who were homozygous for the D allele. In contrast to this, other investigators have suggested a worse response to therapy in patients who carry the D allele (Parving HH 1996). Obviously, large-scale studies are needed to define the impact of genetic factors on the renal protective effects of ACE inhibition.

There are studies suggesting that dual blockade of renin-angiotensin system using a combination of ACE inhibitor and an ARB in patients with nephropathy is superior to the use of either drug alone. (Ayodele OE 2004, Mogensen CE 2000, Rosner MH 2003). The antiproteinuric effects of inhibitors of the renin angiotensin system are increased by sodium restriction and by concomitant administration of diuretics or non-dihydropyridine calcium channel blockers. (American Diabetes Association 2002, Ayodele OE 2004, Arauz-Pacheco C 2002).

In the United Kingdom Prospective Diabetes Study, atenolol showed beneficial effects comparable to captopril on diabetes-related mortality and microvascular complications in patients with type-2 diabetes. (United Kingdom Prospective Diabetes Group 1998) Beta-blockers have been shown to reduce mortality following myocardial infarction, and the absolute benefit of a given relative reduction is greater in diabetics compared to nondiabetics due to higher mortality from myocardial infarction in patients with diabetes. (Ayodele OE 2004) The nondihydropyridine calcium channel blockers have been shown to lower protein excretion in patients with diabetes. (Bakris GL 1990 and 1997) Their antiproteinuric effect may be due to reduction in intraglomerular pressure, reduction in glomerular hypertrophy, and improved glomerular size. The dihydropyridine calcium channel blockers have a variable effect on protein excretion ranging from increased protein excretion to no effect to a fall in protein excretion in various studies. (Melbourne Diabetic Nephropathy Study Group 1991, Vellussi M 1996, Salako BL 2002, Rosssing P, 1997)

14. Protein restriction

The role of dietary protein restriction in CKD is best described as controversial. However, restriction of protein (0.6 g/kg body weight per day) and phosphorus (0.5 to 1 g per day) were shown to reduce the decline in GFR, lower blood pressure, and stabilize renal function compared with higher intakes. This was suggested by a randomized trial involving patients with type-I DM and overt DN. In addition, another study showed that restriction of protein intake to 0.8 g/kg body weight per day reduced the rate of progression to ESRD in patients with type-I diabetes. The National Kidney Foundation recommends that patients with GFR <29 mL/min per m2 should have a daily protein intake of 0.6 g/kg body weight. More recently, a 4-year randomized controlled trial in 82 patients with type 1 diabetes with progressive DN showed that a moderately low–protein diet (0.9 g · kg−1 · day−1) reduced the risk of ESRD or death by 76%, although no effect on GFR decline was observed (Hansen HP, 2002)

A prospective, randomized controlled trial in patients with type 1 diabetes suffering from progressive diabetic nephropathy demonstrated beneficial effect of moderate restriction in dietary protein on the development of ESRD or death. The beneficial effect of protein restriction appeared within the first year, and persisted with continued treatment, as also has been demonstrated in non-diabetic nephropathies suggesting that type 1 diabetic patients with progressive diabetic nephropathy are highly sensitive to dietary protein restriction (Hansen HP, 2002).

The mechanisms by which a low-protein diet may reduce progression of DN are still unknown, but might be related to improved lipid profile and/or glomerular hemodynamics. (Jorge L, 2005) Since diabetic patients have other restrictions to the diet, this may reduce compliance to an additional low-protein diet, although better compliance can be obtained by applying much more intensive dietary counseling (Hansen HP, 2002)

15. Cessation of smoking

Smoking has been shown in many previous studies to effect diabetic complications. Cessation of smoking alone may reduce the risk of progression by 30% in patients with type-2 diabetes. (Ritz E, 2000) Recent studies demonstrated that smokers have increased systolic blood pressures and proteinuria amongst diabetics with nephropathy (Sawicki, PT 1996) More recent work by Chihuran et al has shown that renal function declines faster in smokers than nonsmokers with type 2 DN undergoing treatment to improve blood pressure including ACE inhibitors. Also, loss of renal function is slower in those who stopped smoking. Cigarette smoking remains a risk factor for renal function decline in type 2 DN despite currently recommended therapy (Chihuran T, 2002)

16. Hyperlipidemia

There is suggestion that elevation in lipid levels may contribute to the development of glomerulosclerosis in chronic renal failure. (Ravid M, 1995, Krolewski AS, 1994) Studies have shown that lipid lowering may have a beneficial effect on renal function. (Lam KSL, 1995) A meta-analysis of 13 controlled trials involving a total of 362 subjects, 253 of whom had diabetes, showed that statins decreased proteinuria and preserved GFR in patients with chronic renal disease. (Fried LF, 2001) Adequately powered randomized controlled trials will be needed to determine the role of lipid lowering therapy in retarding the rate of decline in kidney function in patients with chronic renal disease secondary to diabetes mellitus.

17. Renal replacement therapy

The renal replacement modalities available for patients with ESRD from diabetes include peritoneal dialysis, hemodialysis, and renal transplantation. Various studies have shown similar survival in hemodialysis and peritoneal dialysis, though patients are more likely to persist with hemodialysis than with peritoneal dialysis. Both hemo- and peritoneal dialysis limit social life, leisure, and sexual activity.(Hostetter TH, 1981) Patients with diabetes may manifest uremic symptoms at a relatively less-advanced degree of renal insufficiency than their nondiabetic counterparts. (Hostetter TH, 1982)

18. Emerging therapies

Extensive research is currently underway in this field and some new pathogenic mediators for DN have been discovered. These include
• Renin
• Advanced Glycosylation end-products [AGE]
• Protein Kinase C [PKC]
• Transforming growth factor – Beta 1 [TGF-1]
• Nitric Oxide [NO]
• Vascular endothelial growth factor [VEGF]
• Oxidative stress

18.1 Direct renin inhibitor – Aliskiren
Blockade of RAS is a key therapeutic strategy in slowing progression of DN. Interruption of the RAS may also be accomplished by blocking the activity of renin. Aliskiren is a direct renin inhibitor and thus, decreases angiotensin II and aldosterone levels. Aliskiren is a potent antihypertensive and anti proteinuric.

18.2 Advanced Glycosylation end-products [AGEs]
The formation of AGEs and their cross-linked products is a phenomenon of normal aging; however, it is accelerated in the DM. AGE–cross-linked products accumulate in patients with DM and have been implicated in the pathologic process of diabetic complications. Several anti-AGE agents have been tested and shown to be renoprotective in experimental diabetic animal models. (Thomas MC, 2005)
Aminoguanidine is the prototype of an AGE formation inhibitor which acts by scavenging intermediates in the advanced glycation catalytic process.
ALT-946 is a more potent and selective AGE formation inhibitor than aminoguanidine. It has minimal effects on NO synthesis and appears to have fewer toxic effects, although it has not been studied in as much detail.
Pyridoxamine (PYR) - Pyridoxamine is one of the three natural forms of pyridoxine (vitamin B6). It scavenges pathogenic reactive carbonyl species and inhibits the formation of AGEs from Amadori compounds. (Voziyan PA, 2002, Chetyrkin SV, 2008)

18.3 Thiamine
Experimental studies have suggested that thiamine and benfothiamine (S-benzoylthiamine monophosphate), a vitamin B1 derivative, can also prevent or decrease kidney injury. These drugs decrease formation of AGE compounds and protein kinase C (PKC) activity in DN.

AGE Breakers - This group of compounds decreases AGE accumulation by breaking the glycation cross-links.

AGE Receptor Antagonists - AGEs mediate their effects both directly and indirectly through receptor-dependent mechanisms. They bind to the transmembrane receptor for AGE (RAGE) and prevent the development of diabetic microvascular complications. (Bierhaus A 2005, Wendt T, 2003) Thus, RAGE is a potential target to prevent AGE effects.

18.4 Pentoxifylline

Pentoxifylline (PTF) is a methylxanthine derivative with hemorheological properties that has favorable effects on microcirculatory blood flow. In vivo, it also functions as a phosphodiesterase inhibitor. (Navarro J.F., 1999)

18.5 Protein Kinase C inhibitors

Recent studies have identified that activation of PKC initiated by hyperglycemia is associated with many vascular abnormalities in retinal, renal, and cardiovascular tissues. The blocking of PKC-beta isoforms has been shown to decrease albuminuria, structural injury, and TGF-beta expression in animal models of DM. (Koya D, 2000) Ruboxistaurin is one such PKC beta inhibitor.

18.6 Glycosaminoglycans

Glycosaminoglycans are important determinants of GBM permeability. An emerging body of evidence supports the notion that glomerular capillary wall and mesangial alterations in DN involve pathobiochemical alterations of glycoproteins in these structures. Heparin and sulodexide are examples of this class of drugs.

18.7 Endothelin receptor antagonists

DN is associated with enhanced renal synthesis of endothelins. A number of preclinical reports suggested that endothelin might be an appropriate target to decrease DM-related albuminuria. (Turgut F, 2010) Avosentan (SPP301) is a new orally available endothelin 1 antagonist.

18.8 Antifibrotic agents and growth factor inhibitors

Characteristic morphologic lesions of DN include glomerular hypertrophy, thickening of the basement membrane, and mesangial expansion. This leads to glomerulosclerosis, tubulointerstitial fibrosis, and, eventually, loss of kidney parenchyma. Several growth factors which are normally expressed in the kidney have been implicated in the pathogenesis of DN.

TGF-β Inhibitors - Pirfenidone (PFD) is a low molecular weight synthetic molecule that exerts dramatic antifibrotic properties in cell culture and various animal models of fibrosis. SMP-534, another antifibrotic agent is also being studied. Several AGE inhibitors also decrease TGF-β levels. (Turgut F,2010, Sugaru E., 2006)

CTGF Inhibitors - Connective tissue growth factor (CTGF/CCN2) has been associated with fibrosis in various tissues including the kidney. It is up-regulated in most models of DN. Clinical trials evaluating anti-CTGF ab (FG3019) are underway.

18.9 Nitric Oxide (NO) modulation

Abnormalities of renal NO generation have been linked to pathogenesis of renal disease in diabetes. NO and / or NO Synthase are targets for drug development for treatment and/or prevention of DN.

18.10 Vascular endothelial growth factor (VEGF) inhibitors

VEGF is a main regulator of blood vessel growth and plays an important role in promoting endothelial survival and maintaining the microvasculature. Loss of capillaries is strongly associated with the progression of CKD to ESRD (Doi K, 2010).

19. Alternative and complementary therapies for diabetic nephropathy

19.1 Exercise and Yoga

The American Diabetes Association recommends a minimum of 30 minutes of moderate-intensity aerobic physical activity 5 days per week, or vigorous-intensity aerobic physical activity for 20 minutes 3 times per week is recommended for healthy adults aged 18 to 65. Currently, there is no clinical evidence to suggest that vigorous exercise increases the rate of progression of diabetic nephropathy. In fact, some studies have shown that aerobic exercise actually decreased urine protein excretion (Gordon LA, 2008). Additionally, it has been demonstrated that resistance training may have a beneficial effect on muscle mass, nutritional status, functional capacity, and glomerular filtration rate. Therefore, the American Diabetes Association feels that there is no need to restrict exercise in patients with diabetic nephropathy. A more recent study that examined the effects of Yoga and conventional exercise showed findings that suggest better glycemic and blood pressure control obtained in type 2 diabetic patients after Hatha yoga than conventional PT exercises (Gordon LA, 2008).

19.2 Life style modifications

Obesity is often associated with diabetes mellitus and also with nephropathy independent of diabetes (often focal sclerosis). However the impact of weight loss in diabetic subjects with nephropathy on renal function and proteinuria remains as a subject of intense investigation. Short term studies recently reported that weight reduction using dietary therapy for 4 weeks resulted in significant reduction in systolic pressure, proteinuria, and serum creatinine in obese patients with diabetic nephropathy. (A Saiki, 2005). Longer studies involving larger group of patients need to be evaluated to validate such conclusions.

19.3 Herbal and Food derivatives
19.3.1 Curcumin

Curcumin is the active component in Tumeric Rhizomes (Curcuma Long Linn). Curcumin has been shown to possess anti-inflammatory, anti-oxidant and antifibrotic properties in many tissues, in vivo and in vitro studies. Tikoo et al have shown that curcumin treatment prevented the development of DN by significantly lowering blood urea nitrogen and plasma creatinine/body weight ratio in diabetic animals. (Tikoo K, 2008) Various biological actions of curcumin are mediated by inhibiting cell proliferation (Sikora E, 1997), oxidative stress and inflammation (Sharma C, 2006). Several other investigators have also shown that the anti-inflammatory property of curcumin can significantly improve kidney function in animals with chronic renal failure.

19.3.2 Cinnamon

Cinnamon has been known for its antidiabetic effects for some time now. Mishra et al have investigated its effects on nephropathy in diabetes in rodent models of type I diabetes. Histological studies of the kidney proved the protective effect of cinnamon oil by reducing

the glomerular expansion, eradicating hyaline casts, and decreasing the tubular dilatations. (Mishra A, 2010). The authors concluded that the volatile oil from cinnamon contains more than 98 % cinnamaldehyde and that it confers dose-dependent, significant protection against alloxan-induced renal damage. While the mechanism of its action remains unclear, it is believed to be mostly due to its antidiabetic and antioxidant effects leading to reduced formation of AGEs.

20. Conclusions

During the last 3 decades, considerable progress has been made in delaying the progression of CKD even as the frequency of DN continues to increase. This is a truly a reflection of the advances made in understanding the pathogenesis. As reviewed in this chapter many pathogenic cytokines and growth factors have emerged in the recent years that either initiate or contribute to the progressive renal injury in diabetes. Current treatment options are still suboptimal. However with the rapid strides being made in the field, several new therapeutic targets are being recognized and effective treatment strategies being developed.

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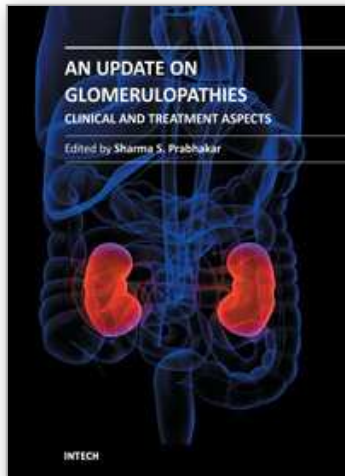
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