Review Of Literature
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A) Clinical Features of Diabetic Nephropathy

Diabetic nephropathy is defined by macroalbuminuria (> 300 mg/day) in the presence of long standing hyperglycemia. Clinically, diabetic nephropathy is characterized by progressive increase in albuminuria (from microalbuminuria, through macroalbuminuria to ESRD), progressive decline in renal function, and hypertension. These clinical elements will be discussed below.

a) Albuminuria

Microalbuminuria, defined as urinary albumin excretion of 150-300 g/day, is an early indicator of incipient diabetic nephropathy. Increased glomerular permeability causes plasma proteins, principally albumin, to enter the ultrafiltrate. While some of this filtered protein is reabsorbed by the proximal tubules, any filtered protein remaining will appear in the urine. Albumin is the most common plasma protein, and the appearance of albumin in the urine is commonly used as an indicator of glomerular damage. Macroalbuminuria is defined as urinary albumin excretion >300 mg/day, and is associated with overt nephropathy. Increasing albuminuria is a clear indicator of progressive renal damage, and is also a strong predictor of non-renal morbidity and mortality, for instance it is the best single predictor of cardiovascular disease progression (Parmar., 2002).

The classical theory of albuminuria, as described above, involves damage to the glomerular membrane and loss of negative charge from the filtration slit resulting in increased filtration of albumin. However, a more recent theory posits that the filtered load of albumin is high even in the healthy kidney, and that the filtered albumin is reabsorbed by the proximal epithelia (Russo et al., 2007). In this modern theory, damage to the glomerular basement membrane and loss of negative charge from the filtration slit plays little or no role in albuminuria (Comper et al., 2008), but rather damage to the tubules causes increased albuminuria (Russo et al., 2009). In either case, albuminuria is an indicator of kidney damage, and a requirement for the diagnosis of diabetic nephropathy.

b) Altered Glomerular Filtration Rate

Glomerular hyperfiltration, i.e. an increase in glomerular filtration rate (GFR), is often seen early in diabetic nephropathy possibly due to increased glomerular capillary pressure and/or leaky glomerular capillaries (Bell et al., 2008; Brands et al., 2008; Miracle et al., 2008; Sochett., 2006;
Thomson et al., 2004), as well as impaired tubuloglomerular feedback (Thomson et al., 2004; Vallon et al., 1999). As the glomerular damage progresses, the thickened capillary basement membrane ultimately decreases the rate of diffusion and results in a decrease in GFR. Additionally, a loss of total number of nephrons contributes to decrease in GFR. A decrease in GFR is the diagnostic criteria for the determination of the severity of kidney damage (Levey et al., 2003). Additionally, reduced GFR is a predictor of non-renal disease, for instance cardiac events (Knobler et al., 2004).

c) Hypertension

Hypertension is one of the clinical hallmarks of diabetic nephropathy (Giunti et al., 2006), as well as a risk factor for developing DN (Shankar et al., 2007). Hypertension in the absence of diabetes is capable of causing kidney damage (Dasgupta et al., 2007; Johnson et al., 2005; Peralta et al., 2005). The most important intervention for slowing the progression of diabetic nephropathy is anti-hypertensive treatment (Schrier et al., 2002; Khosla et al., 2006).

B. Morphological Features of Diabetic Nephropathy

Numerous morphological changes are seen in biopsy or autopsy of kidneys with diabetic kidney disease. Primary changes are the presence of glomerulosclerosis and tubulointerstitial fibrosis, hallmarks of diabetic nephropathy. Glomerulosclerosis is characterized by thickening of the capillary basement membrane, increase in mesangial extracellular matrix (ECM), mesangial cell proliferation, glomerular capillary dilatation, and occasionally glomerular nodules (Figure 1). Tubulointerstitial fibrosis is damage to the renal tubules, and includes tubular atrophy, tubular dilatation, and increased deposition of ECM. Additionally, inflammation and the presence of inflammatory cells are found in both the glomerulus and tubules. Hyperplastic arteriolosclerosis of the afferent arterioles, seen in numerous kidney diseases, is also seen in DN (Hayden et al., 2005).
**a) Glomerular Basement Membrane Thickening**

In diabetic nephropathy, the basement membranes of the glomerular capillaries are thickened (Figure 1), by concentric layers of hyaline material composed primarily of collagen IV (Vinay et al., 2007). Collagen IV is secreted by epithelial cells and is the main component of the basal laminae, along with glycoproteins (e.g. laminin) and proteoglycans (e.g. heparan sulfate). Increased deposition of collagen IV is found in the thickened glomerular capillaries and contributes to tubulointerstitial fibrosis. It is well correlated with renal damage (Dixon et al., 2007; Forbes et al., 2003).

**b) Mesangial Expansion**

In addition to the glomerular basement thickening, expansion of the mesangium is commonly seen in DN (Figure 1). This expansion is caused by both an increase in mesangial cells and an increase in ECM secretion by mesangial cells (Drummond et al., 2002; Tan et al., 2005; Xia et al., 2008). The increase in mesangial expansion is strongly correlated with clinical features of DN, including albuminuria and decreased GFR (Drummond et al., 2002; Tan et al., 2005; Xia et al., 2008).

**c) Microaneurysm (glomerular capillary dilatation)**

An increase in glomerular capillary pressure causes dilatation of the glomerular capillaries (Zatz et al., 1985). This increase in capillary diameter is commonly seen in glomeruli subject to DN (Figure 1), and represents a loss of surface area. Additionally capillary dilatation causes a loss of structural integrity as the dilated capillaries are subjected to greater wall tension. The loss of surface area and structural integrity contributes to both the increase in albuminuria and the decrease in GFR.
d) Inflammation
Chronic inflammation contributes to many chronic diseases (DeLegge et al., 2008; O'Brien et al., 2006; Schottenfeld et al., 2006), including cardiovascular disease (Packard et al., 2008), and is commonly seen diabetic kidney disease (Hou et al., 2004; Kotanko et al., 2006; Macdougall, 2004). Chronic inflammation is characterized by infiltration of inflammatory cells, tissue destruction, and tissue repair including fibrosis. The inflammatory cells are the mononuclear cells of the immune system, including macrophages, lymphocytes, and plasma cells. These inflammatory cells are largely responsible for the tissue destruction, and macrophages in particular direct the tissue repair and fibrosis (Vinay et al., 2007).

![Fig 3: Morphological features of tubulointerstitial fibrosis](image)

e) Tubulointerstitial Fibrosis
In addition to the glomerular damage described above, DN is also associated with tubulointerstitial fibrosis (Figure 3). In fact, this tubulointerstitial fibrosis is better correlated with the extent of kidney damage than glomerulosclerosis (Boor et al., 2007; D'Amico et al., 1998; Nangaku et al., 2004). This observation gives credence to the modern theory of albuminuria, in which tubular damage is responsible for albuminuria rather than glomerular damage (Russo et al., 2007; Comper et al., 2008; Russo et al., 2009). The damaged renal tubules show dilatation and atrophy of epithelial cells. The basement membranes are often thickened, and frank fibrosis is often seen in the interstitium, along with an increase in fibroblasts. This damage severely reduces the tubular
function; in particular proximal tubule reabsorption is reduced contributing to decreased GFR (Thomson et al., 2004).

f) Hyperplastic Arteriolosclerosis
Hyperplastic arteriolosclerosis, which affects the afferent arteriole of the kidney, is a form of arteriosclerosis often seen in kidneys subject to high blood pressure. In hyperplastic arteriolosclerosis, the arteriole walls are thickened by increased layers of smooth muscle, while the lumen is narrowed. This compensation protects the glomerulus by providing a drop in pressure in the afferent arteriole sufficient to prevent intraglomerular hypertension. Unfortunately, the stiffened arteriolar wall and narrowed lumen can also cause both glomerular ischemia and a loss of autoregulation.

C. Pathophysiology of Diabetic Nephropathy
Hyperglycemia is considered the key causative factor in etiology of diabetic nephropathy (DN) (Fig 5). In the presence of high ambient glucose, mesangial cells, endothelial cells, podocytes, and tubular epithelial cells all exhibit maladaptive responses, including excessive ECM, angiotensin II (Ang II) and TGF-β secretion, oxidative stress, as well as apoptosis (Samikkannu et al., 2006). Additionally, hyperglycemia increases the rate of production of advanced glycation end-products (AGEs). AGEs cause dysfunction via interference with normal protein function, as well as activate inflammatory processes via receptors for AGE (RAGEs) (Goldin et al., 2006; Csiszar et al., 2008). Finally, chronic high glucose can cause glucotoxicity through increased production of reactive oxygen species (ROS), which can cause cell dysfunction or increased cellular apoptosis (Han et al., 2008; Susztak et al., 2006). Given this wide range of powerfully damaging mechanisms, the central role of hyperglycemia in the development of DN is without question. As additional evidence for the central role of hyperglycemia, good glycemic control prevents the development of DN. Of course as noted above, most diabetics do not develop DN, so hyperglycemia alone is not sufficient to cause DN. Other contributing factors are hypertension and hyperlipidemia. Albuminuria, which is component of DN, is also an independent risk factor for the progression of DN (Mogensen et al., 1992).

a) Hyperglycemia
Hyperglycemia acts through a variety of mechanisms to cause long term diabetic kidney complications (Brownlee, 2008) (Figure 4). Hyperglycemia increases the rate of non-enzymatic glycosylation and the resulting advanced glycation end-products (AGEs) prevent the normal
functioning of proteins, decrease the turnover rate for proteins, and trigger inflammatory responses. Hyperglycemia also results in accelerated function of normal enzymatic pathways that use glucose as a substrate. This results in deleterious buildup of products, including diacylglyceride (DAG), sorbitol, and hexosamines. Overproduction of ATP via oxidative phosphorylation results in increased production of reactive oxygen species (ROS). ROS, AGEs, and DAG increase Protein kinase C (PKC) activation. For instance PKC activation is increased in mesangial cells exposed to chronic high ambient glucose. These factors stimulate inflammation and local TGF-β production, and result in fibrosis. Indeed, mesangial cells convert to extracellular matrix-overproducing myofibroblasts in response to high ambient glucose and TGF-β. Additionally, hyperglycemia stimulates local Ang-II secretion in mesangial cells, podocytes, and tubular epithelial cells (Miller. 1999; Lansang et al., 2002).

Fig 4. Mechanisms of hyperglycemic damage.
b) Angiotensin II (Ang-II)

The systemic role of Ang-II in maintaining blood pressure is well known. However, the intrarenal function of Ang-II possibly plays a more important role in the progression of DN (Liu et al., 2008). As mentioned above, hyperglycemia stimulates the local synthesis of Ang-II and enhanced intrarenal Ang-II is involved in the deterioration of renal function (Yamamoto et al., 2007). Ang-II stimulates production of TGF-β, and adversely alters renal hemodynamics, principally by causing efferent arterial constriction. Blockade of the Ang-II production by use of angiotensin converting enzymes inhibitors (ACEI) or Ang-II receptor blockers (ARBs) significantly slows the progression of DN, even in the absence of hypertension. Additionally, urinary angiotensinogen, a marker of the intrarenal RAS and Ang-II, is increased in hypertensive patients, and is decreased by RAS blockade (Kobori et al., 2009).

c) Hemodynamic Factors

Increased systemic blood pressure, loss of local autoregulation, and constriction of the efferent arteriole can each independently cause an increase in glomerular capillary pressure (GCP). If multiple problems with pressure regulation occur simultaneously, the increase in GCP is even greater. Increased GCP produces a mesangial stress and provokes the secretion of TGF-β and accompanying secretion of ECM by mesangial cells (Cortes et al., 1999). This response by mesangial cells is exaggerated by high glucose and Ang-II. Additionally, increased GCP is thought to produce increased GFR and microalbuminuria seen early in the development of DN.

d) Transforming Growth Factor-β (TGF-β)

TGF-β secretion is stimulated by mesangial stretch, hyperglycemia, inflammation, and Ang-II. TGF-β has a wide array of biological activities, including controlling cell proliferation and apoptosis, stimulating ECM accumulation and angiogenesis, and regulating effects of other cytokines (Schrijvers et al., 2004; Sharma et al., 1996). The role of TGF-β in response to glomerular damage is to stimulate the synthesis and inhibit the degradation of ECM. This increase in ECM is an appropriate response to acute injury; however, with chronic hyperglycemia, the response becomes maladaptive and results in fibrosis. The increase in TGF-β and its accompanying fibrosis are central to both glomerulosclerosis and tubulointerstitial fibrosis seen in DN (Kanwar et al., 2008; Border et al., 1994).
e) Albumin and Total Proteins
As mentioned above, albuminuria is a component of DN and is also an independent risk factor for progression of DN (Locatelli et al., 1996; Peterson et al., 1995). Protein that escapes into the ultrafiltrate can be reabsorbed by the epithelial cells of the proximal tubules. However, in the classical theory of albuminuria, as the glomerular membrane becomes leaky and the filtered load of protein increases, the reabsorbed protein becomes harmful (Stevens et al., 2006). When exposed to albumin, epithelial cells of the proximal tubule produce matrix proteins and TGF-β (Diwakar et al., 2007), and potentially undergo an epithelial mesenchymal transformation (Bagnasco et al., 2009). Consequently, an increase in albuminuria is an indicator of renal decline, while reduction in albuminuria is an appropriate therapeutic goal in management of kidney disease (Wilmer et al., 2003). It is also interesting to note that once damage becomes sufficiently severe, glycemic control is unable to prevent further decline. For instance, albuminuria can cause additional damage without hyperglycemia.

f) Inflammation
As mentioned above, hyperglycemia can cause inflammation by increased ROS production and through AGE-RAGE interactions. Chronic hyperglycemia leads to chronic inflammation and the recruitment of monocytic immune cells, e.g. macrophages and lymphocytes. CD68-positive cells, a marker of activated macrophages, accumulate in chronically inflamed tissues. Transforming growth factor-β (TGF-β) is secreted by activated macrophages and stimulates collagen expression by neighboring cells. CD68-positive activated macrophage infiltration and TGF-β expression are markers of damaged diabetic kidneys (Dixon et al., 2007; Forbes et al., 2003; Dalla Vestra et al., 2005). Interleukin 6 (IL-6) is a pro-inflammatory cytokine that is secreted locally by macrophages and T-cells (as well as circulating systemically). IL-6 has also been shown to be increased in the damaged kidney (Navarro et al., 2005).

g) Vascular endothelial Growth Factor
VEGF and VEGF receptor expression is critical to maintaining normal glomerular podocyte and renal tubular function. Renal podocytes are the key regulators of macromolecule permeability and are also the major synthesizers of VEGF. VEGF expressed in glomerular podocytes activates VEGF-R2 on glomerular capillary endothelial cells, maintaining and regulating the endothelial fenestrations and permeability in a similar manner to its role in the
ocular choriocapillaris (Schrijvers et al., 2004). Elevated VEGF levels increase glomerular permeability and contribute to diabetic proteinuria, leading authors to hypothesize that high glucose levels (working through reactive oxygen species) elevate VEGF, which in turn increases glomerular permeability and, therefore, contributes to proteinuria (Lee et al., 2006).

h) Adiponectin

Adiponectin, an adipocyte-derived protein, is actually present in serum as a trimer, hexamer or highmolecular weight (HMW) form (Kadowaki et al., 2005; Whitehead et al., 2006) [1,2]. Accumulating evidence has demonstrated that circulating adiponectin has a number of vascular protective qualities such as insulin sensitizing, anti-inflammatory and anti-atherogenic effects (Shimada et al., 2004). One potential pathway by which adiponectin and AMPK activation may provide protection against albuminuria and podocyte permeability is via reduction of oxidant stress (Ouedraogo et al., 2005; Tao et al., 2007; Alba et al., 2004). Nox4 is a recently described nonphagocytic NAPDH oxidase that is highly expressed in the kidney (Geiszt et al., 2000) (30). Podocytes expresses Nox4 and adiponectin and AMPK regulate Nox4 protein in podocytes. Oxidant stress has been consistently linked with insulin resistance, obesity, and adiponectin deficiency. The kidney’s contribution to oxidant stress has been largely ignored in settings of insulin resistance. The systemic adiponectin deficiency causes upregulation of Nox4 in the kidney and podocytes. Adiponectin-AMPK-Nox4 pathway has been shown to increase albuminuria. Over-expression of adiponectin has beneficial effects on early stage diabetic nephropathy (Nakamaki et al., 2011).

i) Erythropoietin

Erythropoietin (Epo) is an endogenous glycoprotein stimulating erythrocytosis, which interacts with erythroid progenitor cells to promote their proliferation and maintain their viability as they differentiate (Koury & Bondunrant, 1990; Spivak, Pham, Isaacs, & Hankins, 1991). Epo is produced in the interstitial cortical cells of the kidney, and anaemia due to low Epo levels is typically observed in patients with renal failure, as soon as glomerular filtration falls below 40 mL/min (Radtke et al., 1979).
Fig 5: EPO in Kidney: Increased EPO synthesis in kidney predisposes it to progressive kidney disease

j) Glomerular Filtration Rate
The ideal marker of glomerular filtration rate (GFR) should be an endogenous molecule which, being produced at a constant rate, is cleared solely by the kidneys via free glomerular filtration, without being neither secreted by tubular cells, nor reabsorbed into peritubular circulation. Plasma concentration of creatinine is the most commonly used test to evaluate an impairment of GFR. However, creatinine does not completely fulfill the characteristics of an ideal marker of GFR. In fact, besides glomerular filtration, creatinine is also secreted by renal tubules. Furthermore, at least in patients with advanced renal failure, creatinine is also eliminated by extra-renal clearance. Many low molecular weight (MW) proteins are cleared from the plasma mainly by the kidneys via glomerular filtration, followed by complete tubular reabsorption and complete catabolization inside tubular cells. Due to such renal handling, the measurement of plasma concentration of various low MW proteins has been proposed as a useful tool to evaluate an impairment of GFR. Furthermore, published data suggest that some of these proteins, namely cystatin C and β2-microglobulin, could be better markers of GFR than creatinine. (Donadio et al., 2001)

k) Other Vascular Problems
Intrarenal Ang-II is elevated in hyperglycemia, leading to increased glomerular capillary pressure (GCP). Additionally, hypertension is a component of DN, and an independent risk factor for
disease progression. Increased myogenic tone in the afferent arteriole protects the glomerulus from hypertension, but chronic hypertension leads to hyperplastic arteriolosclerosis (Khavandi et al., 2009). With arteriolosclerosis comes a loss of autoregulation and increased risk of ischemia.

**h) Podocytopathy**

Although the traditional view of diabetic glomerulosclerosis and its resulting albuminuria has focused on the role of mesangial cells, recent work suggests that damage to the podocyte is actually more important to development of albuminuria (Menne et al., 2006; Su et al., 2007; Wolf et al., 2005). Hyperglycemia can cause apoptosis in podocytes (Susztak et al., 2006) as well as loss of podocyte foot process proteins. For instance, nephrin, a structural protein of the podocytes foot process, is decreased in DN, contributing to the loss of filtration barrier integrity. (Wolf et al., 2005). The loss of nephrin appears to result from hyperglycemia. (Menne et al., 2006)
Fig 6: Schematic representation of mechanism of different manifestations of diabetic nephropathy.
D. Treatment of Diabetic Nephropathy

If left untreated, diabetic nephropathy is a disease with a high rate of mortality and morbidity, but with proper detection and treatment, DN can be avoided or delayed (Trocha et al., 1997). Aggressive management by inhibiting the pathological pathways described above can greatly improve the outlook for these patients (Foggensteiner et al., 2001).

a) Intensive Glycemic Control

Given that hyperglycemia is the keystone that is responsible for initiating the deleterious events of diabetes, it is only reasonable that intensive glycemic control delays the onset and slows the progression of diabetic nephropathy as well as other diabetic complications, e.g. retinopathy and neuropathy (The Diabetes Control and Complications Trial Research., 1993). Intensive glycemic control is most effective when started early. When near normal glycemia is maintained in normoalbuminuric patients, prevention of nephropathy is possible (Ruggenenti et al., 2000). However, when intensive glycemic control is started after incipient or overt nephropathy, results are discouraging (Ruggenenti et al., 2000). Once sufficient albuminuria is present, pathological processes are underway that no longer require hyperglycemia, and the disease process becomes self-sustaining.

b) Renin-angiotensin system (RAS) Blockade

ACE inhibitors (ACEIs), which include captopril (Capoten®), enalapril (Vasotec®), ramipril (Altace®), as well as many others, inhibit angiotensin converting enzyme (ACE). Angiotensin receptor blockers (ARBs), which include candesartan (Cilexetil®), losartan (Cozaar®), valsartan (Diovan®), are antagonist to angiotensin II (Ang-II) receptors. Both ACEIs and ARBs block the renin-angiotensin system (RAS). Elevated systemic Ang-II can cause hypertension. While hypertension is an independent risk factor for progression to DN, and RAS blockade is a cornerstone in the treatment of hypertension, the benefits of ACEIs and ARBs in treating DN are independent of their antihypertensive effects (Radbill et al., 2008; Stump et al., 2006; Zhang et al., 2008). Blockade of RAS by use of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) has proven useful in slowing the progression of DN (Brenner et al., 2001; Lewis et al., 1993; Lewis et al., 2001). RAS mediates kidney damage through a wide range of mechanisms and the actions of RAS blockade provide benefits through pleiotropic mechanisms including, antihypertensive, hemodynamic, and antiproteinuric effects (Zhang et al., 2008; Ruilope. 2008). ACEIs and ARBs are the first line anti-hypertensive drugs for diabetic patients. RAS blockade has been shown to decrease albuminuria and limit progression of
DN (Brenner et al., 2001; Lewis et al., 1993; Lewis et al., 2001; Chiurchiu et al., 2005), but it has not been shown to reverse DN (Brenner et al., 2001; Lewis et al., 1993; Lewis et al., 2001). Therefore, RAS blockade is most useful early in DN; however, the benefit of RAS blockade in non-proteinuric patients is unclear. Additionally, ACEIs and ARBs reduce the risk of developing diabetes, while both thiazide diuretics and β-blockers accelerate the appearance of new-onset T2D in patients with hypertension. Ang-II decreases mitochondrial content of skeletal muscle and blockade of RAS helps to preserve insulin sensitivity by preserving skeletal muscle mitochondria. Additionally, recent studies have identified that ACEIs and ARBs also attenuate AGE accumulation. It is postulated that ACEIs and ARBs reduce the accumulation of AGE in diabetes partly by increasing the production and secretion of soluble RAGE into plasma (Forbes et al., 2005).

c) Other Anti-hypertensives
Hypertension is both a component of DN and an independent risk factor for DN progression. Reduction of blood pressure is beneficial in preventing progression of DN. As mentioned above, ACEIs and ARBs exhibit benefits in DN that are independent of their anti-hypertension effects, and are therefore the first line anti-hypertensive medicines for DN. Additionally, other anti-hypertensive medications including thiazide, loop, and K+ sparing diuretics, as well as aldosterone blockers, calcium channel blockers, and β-blockers. Achieving appropriate blood pressure is more important than the agents used, and often more than one anti-hypertensive agent is needed. For patients with albuminuria < 1 g/d, the goal blood pressure is 130/80 mmHg, while for patients with albuminuria > 1 g/d the goal pressure is 125/75 mmHg (Peterson et al., 1995).

d) Diet Modification: Low Sodium, Low Protein
Dietary sodium reduction has been shown to reduce blood pressure, and prevent the onset of hypertension (Lin.2009; Sacks et al., 2001). Moreover, maintaining high dietary potassium consumption is beneficial for preventing or reducing hypertension (Cook et al., 2009), but high potassium diets are contraindicated in patients with renal failure. Some studies suggest that dietary protein restriction can prevent or reduce the progression of diabetic and non-diabetic kidney diseases (Hansen et al., 2002; Pedrini et al., 1996; Uribarri et al., 2006), while other studies do not find a benefit for low protein diets (Klahr et al., 1994; Meloni et al., 2002).