REVIEW ARTICLE

Diabetic Nephropathy: Progression and Pathophysiology

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ABSTRACT

Diabetic nephropathy is the leading cause of kidney disease in patients starting renal replacement therapy and affects ~30% of type 1 and type 2 diabetic patients. This review focuses on the progression and pathophysiological aspects of the condition. The natural history of diabetic nephropathy is characterized by specific renal morphological and functional alterations. Features of early diabetic renal changes are microalbuminuria (30-300mg/day), glomerular hyperfiltration, glomerular and renal hypertrophy, increased basement membrane thickness, and mesangial expansion with the accumulation of extracellular matrix proteins such as collagen, fibronectin, and laminin. Advanced diabetic nephropathy is characterized by macroalbuminuria (>300mg/day), a progressive decline in glomerular filtration rate, decreasing creatinine clearance, glomerulosclerosis, and interstitial fibrosis. Although poor glycemic control is an important risk factor, glycemia does not fully explain why only a subset of diabetic patients progress to end stage renal disease. Several decades of extensive research has elucidated various pathways to be implicated in the development of diabetic kidney disease such as systemic and glomerular hypertension, advanced glycation end-products and the aldose reductase system. Furthermore, hemodynamic factors, the renin-angiotensin system, the endothelin system, the intracellular signaling molecule protein kinase C, transforming growth factor-ß, growth hormone, insulin like growth factors, vascular endothelial growth factor, and platelet-derived growth factor are believed to be involved in the pathogenesis. Thus, there are clearly many points at which therapeutic approaches could be tried to provide renoprotection in diabetes. It is likely that due to its complexity, targeting multiple points in altered metabolism in the diabetic kidney will be more successful in attenuating the development of diabetic nephropathy, rather than a single approach.

Key Words: Diabetic Nephropathy; Hyperglycemia; Microalbuminuria; Pathophysiology

INTRODUCTION

Diabetes Mellitus is a major contributor to morbidity and mortality in society. It is the leading cause of blindness in adults, the most important reason for amputation after trauma, the most important risk factor for atherosclerotic vascular disease, and the most frequent cause of end-stage renal disease. Diabetes mellitus has recently assumed epidemic proportions and affects more than 285 million individual worldwide. Global estimates for the year 2030 predict a further growth of almost 50%, with the greatest increases in the developing countries of Africa, South America and Asia.[1] Currently India leads the world with the largest number of diabetic subjects and this is expected to further rise in the coming years. Given the high prevalence of diabetes in Indians with over 50 million diabetics already and the numbers expected to increase to 87 million by the year 2030[1], this could place considerable burden on the health budgets of this country.

The medical and socio-economic burden of the disease is caused by the associated

complications, which impose enormous strains on health-care system. The devastating complications of diabetes are mostly macrovascular and microvascular disease. Microvascular complications, including nephropathy, develop some years after the onset of diabetes. Genetic background is likely to be important in determining susceptibility to diabetic nephropathy, but exposure of tissues to chronic hyperglycemia is the main initiating factor. Diabetic nephropathy is major microvascular complication of diabetes, a leading cause of end-stage renal disease and is associated with increased cardiovascular mortality.[2] Diabetic nephropathy is a clinical syndrome characterized by the occurrence of persistent albuminuria in concomitance with type 1 and type 2 diabetes.

The description of albumin in the urine as a sign of serious kidney diseases in 1836 by Bright[3], physician to Guy's Hospital, marked the advent of clinical nephrology. This observation, together with earlier ones by Cotunnius[4] in 1770 and Rollo[4] in 1798 that urine of some diabetics contained proteins, led Rayer[5] in 1840 to postulate that diabetes might cause a form of "Bright's disease". Indeed various epidemiologic studies[6,7,8] have demonstrated that about 20-40% of diabetic subjects will develop proteinuria and progressive renal failure on an average of 15-20 years after the onset of diabetes. The prognosis of these patients is poor and without renal support therapy, the mean survival after the onset of clinical proteinuria is only 5 year.[9] Aside from the personal and domestic tragedy, the cost of caring for the diabetic patient in end stage renal disease is enormous. Analyses of the WHO Renal Data System demonstrated a dramatic increase in the incidence of ESRD that is caused by diabetes.[10] Between 1999 and 2005 diabetes was responsible for > 44% of all new cases of ESRD. Thus, whereas the population with diabetes grew 40% between 1984 and 1996, the number of people who initiated treatment for ESRD as a result of diabetes increased by 400%. Between 1996 and 2005, the annual incidence grew by another 37%. On the basis of these data, it is expected that the burden of diabetic nephropathy will increase further in the next years, although perhaps at a less accelerated growth rate. Caring for these patients is a formidable task since they are usually beset by numerous other diabetic complications. Successful management requires an understanding of the molecular mechanism and natural history of the condition.

NATURAL HISTORY OF DIABETIC NEPHROPATHY

The progression of nephropathy in type 1 diabetes has classically been described as a series of stages in a relentlessly deteriorating course from normal renal function to end stage renal disease marked by increasing amounts of albuminuria.[11] At the time of initial diagnosis there are no significant renal histologic abnormalities, and renal plasma flow (RPF) and glomerular filtration rate (GFR) are elevated. Within 3 years, histologic changes (increased measangial matrix material and glomerular basement membrane thickening) of diabetic nephropathy are evident but GFR and RPF remain elevated. Over the subsequent 10-15 years there is progressive histologic damage but renal hyperfiltration persists, approximately 15 years after the diagnosis of diabetes, albuminuria (> 300 mg/day; overt albuminuria, macroalbuminuria) is detected and the elevated rates of RPF and GFR have returned to normal. This is an ominous sign and heralds the onset of progressive renal insufficiency. At this stage no intervention has been shown to slow the rate of decline of GFR. Within 5 years of the onset of albuminuria approximately half of the individuals will have experienced a 50% reduction in the GFR and a doubling of their serum creatinine. Within a mean of 3 to 4 years, half of these patients will have progressed to ESRD. At or just before the time of onset of overt albuminuria, most patients will develop hypertension and the increase in blood pressure markedly accelerates the progression of renal disease.[11] Effective treatment of the hypertension has been documented to slow, although not prevent, the progression to ESRD. Once clinically significant albuminuria (> 300 mg/day) has developed, tight glycemic control cannot prevent the development of renal insufficiency.[12]

In the early 1980's independent investigators[13] reported that there is a "preclinical" stage of diabetic nephropathy, characterized by urinary albumin excretion rate that are not detectable by standard laboratory methods and termed this microalbuminuria (Table 1). Normal individuals do not excrete more than 10-15 mg/day of albumin. However, routine laboratory tests do not detect these small amounts of albuminuria unless it is in excess of 300 mg/day, that is distinctly abnormal, yet cannot be detected by routine means. This range (30-300 mg/day or 20-200 µgram/min) has been referred to as microalbuminuria and is the first laboratory evidence of diabetic renal disease. Fortunately, microalbuminuria can be detected using more sophisticated techniques (Radioimmunoassay, Enzyme linked immunosorbant assay) and a highly accurate screening urine test for microalbuminuria now exists (Micral, Boehringer, Mannheim). Initial retrospective studies[14,15] reported that approximately 80% of patients with type 1 diabetes would progress from microalbuminuria to overt albuminuria over 6 to 14 years. Because the post hoc values for microalbuminuria that are predictive of progression to overt albuminuria differed among these initial investigations, a conference was convened to achieve consensus on the definition of microalbuminuria[16] and these values have been used up to the present time (Table 1). More importantly, tight glycemic control with insulin during the microalbuminuria stage has been shown to prevent the development of overt diabetic nephropathy. [17]

Table-1: Definitions of Abnormal Albumin Excretion

	Urinary AER (mg/day)	Urinary AER (µg/min)	Urine Albumin: Creatinine Ratio (mg/mg)
Normal	< 30	< 20	< 0.02
Microalbuminuria	30 – 300	20 – 200	0.02 – 0.20
Macroalbuminuria	> 300	< 200	> 0.20

AER: Albumin Excretion Rate

The natural history of diabetic nephropathy in type 2 diabetes follows that in type 1 diabetes with the exception that microalbuminuria stage may be present at the time of diagnosis, reflecting the fact that most people with type 2 diabetes mellitus have had hyperglycemia for a number of years before diagnosis. Taken together, a series of stages in the development of renal changes in diabetes is recognizable:

Stage 1: Early hypertrophy and hyperfunction
Stage 2: Glomerular lesion without clinical disease
Stage 3: Incipient diabetic nephropathy/ microalbuminuria stage: urine albumin excretion 30-300mg/day
Stage 4: Overt diabetic nephropathy/ macroalbuminuria stage: urine albumin excretion $\geq$ 300 mg/day
Stage 5: End-stage renal disease

It has been appreciated for more than a decade that microalbuminuria predicts risk for progression to overt albuminuria in both type 1 and 2 diabetes.[18,19,20] It is an early warning system to alert clinicians to intervene at a time when future renal damage is still preventable. In type 2 diabetes, some might argue that the predictive value of microalbuminuria is less but most agree that it still indicates a need for appropriate evaluation and treatment. Further, a number of studies have shown that once proteinuria occurs, the decline in renal function continues at the same rate regardless of the type of diabetes and hence microalbuminuria is regarded as marker of progression of diabetic nephropathhy.

COMORBID ASSOCIATIONS AND RISK FACTORS

In addition to higher blood pressure and premature cardiovascular disease, diabetic patients with nephropathy have more neuropathy, more retinopathy, more marked insulin resistance and left ventricular hypertrophy and dysfunction than diabetic individuals with normal albumin excretion. These abnormalities tend to worsen as proteinuria rises. The two factors most important in the initiation and progression of nephropathy are blood glucose and blood pressure. Dyslipidemia and smoking may be deleterious, although hard evidence is lacking. The gradual impairment of laboratory findings

is caused by structural alterations at the renal level, which at the beginning consist of a gradual and progressive accumulation of extracellular matrix in the mesanglum and glomerular basement membrane. Later the formation of mesangial nodules represents the characteristic lesion of the Kimmelstell – Wilson nephropathy with additional extensive tubulointerstitial lesions. Morphological lesions of varying degree are present in over 90% of type 1 diabetes[21], whereas clinical nephropathy develops in only 20-40%.[22] At present there is no early morphological appearance which will distinguish those patients who are at risk from those who are not. Indeed, this distinction may never be found and the difference may simply be quantitative, patients in whom the most nephrons are damaged are those who will be affected by clinical nephropathy. Identification of patients prone to develop the clinical disease will be primarily based on functional parameters. It is therefore important to describe the early functional anomalies, recognize their origins and determinants, examine their relationship to diabetes and its control and analyze their prognostic significance. Table 2 lists the risk factors implicated in the development of microalbuminuric stage.

Table-2: Risk Factors for Diabetic Nephropathy

Hyperglycemia	Male Gender	Hypertension
Long Duration of Diabetes	Cigarette Smoking	Genes • Insulin Resistance • Angiotensin Converting Enzyme • Ion Transport Dysregulation • Hypercholesterolemia
Family History • Hypertension • Cardiovascular Disease • Nephropathy	Ethnicity • Mexican-American • Native–American • African-American • Asian	

PATHOPHYSIOLOGY (ORIGIN AND DETERMINANTS OF MICROALBUMINURIA)

It is generally believed that the increased urine albumin excretion in diabetic nephropathy is mostly glomerular in origin. For albumin to appear in the urine it must cross the glomerular filtration barrier, which consists of fenestrated glomerular endothelial cells, the glomerular basement membrane, and the glomerular epithelial cell or podocyte. It has long been appreciated that increased intraglomerular pressure, loss of negatively charged glycosaminoglycans in the basement membrane and, later in the disease process, increased basement membrane pore size, all contribute to the albuminuria. Loss of anionic charge in biochemical terms means loss of normal heparan sulphate proteoglycan, the main glycosaminoglycan component of basement membranes of glomeruli.[23] There is a lot of evidence to support this:

1. The loss of heparan sulphate in the GBM leads to loss of anionic sites and albuminuria.[24]
2. Loss of heparan sulphate in the GBM has been demonstrated in diabetic patients with nephropathy.[25]
3. The multiplicity of the effects of heparan sulphate proteoglycan might well explain the strong association between vascular dysfunction and renal and extrarenal complications. Heparan sulphate proteoglycan not only inhibits the glomerular filtration of albumin but also contributes to the integrity of the pore size of the GBM. Thus, loss of heparan sulphate proteoglycan leads to disruption of the microstructure of the GBM.[26] Heparan sulphate proteoglycan also strongly inhibits mesangial cell growth, and loss of heparan sulphate has been shown to be an inducer of the mesangial expansion.[27]
4. Finally, diabetes affects heparan sulphate metabolism and leads to loss of normally sulphated heparan sulphate in extracellular matrix and plasma membranes.[23] Usually heparan sulphate is sulphated within the golgi apparatus of many cells. The key enzyme of sulphation is N-deacetylase. After sulphation has taken place, heparan sulphate is incorporated into plasma and basement membranes where it contributes to the anionic charge of the extracellular matrix and integrity of the collagen network. [26] Loss of anionic charge in GBM and increased glomerular permeability due to decreased sulphation of glomerular heparan

sulphate has been described in experimental membranous nephropathy.[24] In diabetic animals inappropriate sulphation of heparan sulphate has been demonstrated probably due to impaired activity of the deacetylase enzyme. [28]

Therefore, it is hypothesized that (micro)albuminuria and the associated complications are due to genetic polymorphism of enzymes involved in the metabolism of heparan sulphate proteoglycan (e.g. N-deacetylase). Genetic polymorphism of diabetes – sensitive enzymes involved in the metabolism of glycosaminoglycans has been demonstrated in rats.[29] Accordingly, patients who develop (micro) albuminuria are characterized by iso-enzymes which are extremely vulnerable to poor diabetes control. In these patients a critical reduction of normal heparan sulphate would be expected, leading to albuminuria and progression of mesangial expansion, retinopathy and macroangiopathy, whereas persons equipped with iso enzymes less vulnerable to hyperglycemia would be protected. Thus, the polymorphism of enzyme involved in the metabolism of heparan sulphate proteoglycan might be the reason for the heterogenous prognosis of poorly regulated diabetic patients and for the fact that only subset of diabetic patients develop microalbuminuria.

Well described microscopic abnormalities include thickening of the glomerular basement membrane, accumulation of mesangial matrix, and increased numbers of mesangial cells. As disease advances, there is a close relationship between mesangial expansion and declining glomerular filtration.[30] Mesangial expansion also correlates inversely with capillary filtration surface area, which itself correlates to glomerular filtration rate.

Changes in the tubulointerstitium, including thickening of the tubular basement membrane, tubular atrophy, interstitial fibrosis and arteriosclerosis, are also well described. Interstitial enlargement correlates with glomerular filtration, albuminuria, and mesangial expansion. It has been suggested that the accumulation of protein in the cytoplasm of proximal tubular cells causes an inflammatory reaction which leads to tubulointerstitial lesions.[31] Recently, it has been demonstrated that the podocytes also have a role in increasing proteinuria and developing glomerulosclerosis. The podocyte is a terminally differentiated epithelial cell with a cell body from which numerous processes branch.[32] These processes divide successively until the terminal foot process rests on the glomerular basement membrane. Foot processes interdigitate so that neighbouring foot processes are from different podocytes. The podocyte, via the foot processes, provides structural support for the glomerular capillaries, buffers intraglomerular pressure, and is the final layer in the barrier to protein passage across the glomerulus into the urinary space. Like the basement membrane, the podocyte is covered by negatively charged molecules, which help repel anionic proteins such as albumin. In addition, the negative charge helps maintain open the slit diaphragm, the structure which bridges the gap between adjacent foot processes. The slit diaphragm is essential in preventing proteinuria, slit diaphragm proteins such as nephrin having an essential role in preventing escape of protein into Bowman's space.

In both human and experimental diabetes, podocyte morphology is abnormal.[33] The foot processes broaden and efface. Eventually there is loss of the podocyte itself. Podocytes cannot regenerate so this loss cannot be compensated for. Also, there is decreased expression of nephrin mRNA and protein.[34] Abnormalities in several podocyte proteins have been demonstrated to cause proteinuric renal diseases in humans, for example: absence of nephrin in Finnish congenital nephritic syndrome; CD2-adaptor protein and podocin in forms of steroid resistant nephritic syndrome. Thus it is probable that podocyte protein abnormalities in diabetes contribute to proteinuria and eventual glomerulosclerosis. Whether these are primary abnormalities in the development of proteinuria in diabetes, or occur later in the disease process is a matter of some controversy currently.

FROM HYPERGLYCEMIA TO DIABETIC KIDNEY DISEASE: THE ROLE OF METABOLIC, HEMODYNAMIC, INTRACELLULAR FACTORS AND GROWTH FACTORS / CYTOKINES

Genetic background is likely to be important in determining susceptibility to diabetic nephropathy, but exposure of tissues to chronic hyperglycemia is the main initiating factor. A number of potential mechanisms exist by which poor glycemic control can damage the kidney and initiate or propagate the development of diabetic nephropathy. Along with the role of high blood glucose, the pathophysiological role of different metabolic pathways in the development and progression of diabetic nephropathy has been suggested which includes:

I. Growth Factor and Cytokines
a) Transforming Growth Factor β (TGF- β)
b) Platelet – Derived Growth Factor (PDGF)
c) Connective Tissue Growth Factor (CTGF)
d) Growth Hormone (GH) and Insulin like Growth Factors (IGFs)
e) Vascular Endothelial Growth factor (VEGF)
II. Hemodynamic Factors
a) Angiotensin II (Ang II)
b) Endothelin (ET)
III. Metabolic Factors
a) Advanced Glycation End Products (AGEs)
b) Aldose Reductase (AR) / Polyol Pathway
IV. Intracellular Factors
a) Diacylglycerol (DAG) – Protein Kinase C (PKC) Pathway

Resident and non-resident renal cells are stimulated by hyperglycemia in producing humoral mediators, cytokines, and growth factors that are responsible for structural alterations such as increased deposition of ECM and functional alterations such as increased permeability of glomerular basement membrane or shear stress. These alterations contribute to diabetic nephropathy. Glucose influx in the renal cells is modulated by GLUT-1, which is a surface receptor of resident renal cells. Heilig et al[35], demonstrated that in vitro, high glucose concentrations (400 to 550 mg/dl) induced over expression of GLUT-1 mRNA and over production of GLUT-1 protein in mesangial cells. In addition, glucose transport increased in cells. GLUT-1 is modulated in its expression by TGF-β_1. In fact, Inoki et al[36], demonstrated that this growth factor modulation was dose and time dependent. When an anti – TGF-β_1 monoclonal antibody was added in vitro, GLUT-1 mRNA expression and D-glucose uptake was reduced. In conclusion, endogenous TGF-β_1, produced by mesangial cells cultured under high-glucose conditions, is able to enhance glucose transport to stimulate glucose uptake by inducing the over expression of mRNA and protein GLUT-1. Thus, it accelerates glucose induced metabolic abnormalities in mesangial cells.

Another growth factor, PDGF-β is involved in structural alterations at the glomerular level. Dipaolo et al[37], demonstrated in vitro down regulation of TGF-β_1 in human mesangial cells in the presence of high glucose concentration and anti-PDGF BB neutralizing antibody. They evidenced that a high glucose concentration induced an early and a persistent increase of PDGF-B chain gene expression, whereas PDGF-β receptor mRNA increased by two fold after 6 hrs, thereafter declining after 24 hrs. In contrast, TGF-β_1 mRNA increased after 24 to 48 hrs of incubation in high glucose. Therefore, they concluded that high glucose induces an early activation of a PDGF loop that in turn causes an increase of TGF-β_1 gene expression, thus modulating both human mesangial cell proliferation and mesangial matrix production. Connolly et al[38] demonstrated that another growth factor, connective tissue growth factor, plays an important role in glomerular alteration in diabetic sclerosis because this mediator induces transient actin cytoskeleton disassembly in mesangial cells, high production of fibronectin, collagen types I and IV, and mesangial cell hypertrophy. Finally, angiotensin II is an additional growth factor that stimulates resident renal cells to produce TGF-β_1. In addition, angiotensin II is generated in hypertension, a disorder that frequently accompanies diabetes and accelerates progression of diabetic nephropathy. In vitro studies have shown that angiotensin II increases ECM accumulation by mesangial cells, primarily via stimulation of TGF-β expression.[39]

Classically, growth hormone (GH) secreted from the pituitary induces the synthesis of Insulin-Like Growth Factor (IGF) in various tissues through activation of GH receptor (GHR). Two lines of evidence implicate GH in the pathogenesis of glomerular fibrosis in experimental diabetes. One is the renoprotective effect of long-acting somatostatin analogs and GHR antagonists.[40] The other is the failure of streptozotocin (STZ) treated mice, which are either transgenic for a mutated GH, which is an antagonist of native GH, or in which the GHR / binding protein gene has been knocked out, to develop glomerular lesions.[41] Transgenic mice that over express GH or GH releasing factor develop glomerulosclerosis, but those expressing an IGF-1 transgene have morphologically normal, though enlarged, glomeruli.[42] Overall the evidence favors GH having a direct effect in the pathogenesis of glomerulosclerosis, independent of IGF. Plasma GH levels are raised in type 1 diabetic patients with poor glycemic control, while the concentration of IGF-1 is low and that of IGF binding protein (IGFBP1), a modulator of its activity is raised.[43] IGF-1 may be expressed in the kidney independently of GH. MC (Mesangial cells) cultured on glycated-albumin increase IGF-1 production, and the growth factor directly stimulates synthesis of laminin, fibronectin, proteoglycan, and type IV collagen in these cells.[44] IGF-1 and IGF-BP species have also been reported to increase in the kidney in the early stages of experimental diabetic nephropathy.[40] In the presence of high glucose, IGF increased IGF-1 stimulated insulin receptor substrate - ½ phosphorylation and AP-1 transcriptional activity, while decreasing IGFBP-2 expression. Thus, despite lower circulating levels of IGF-1 in diabetes, locally produced growth factor may influence in vivo. MC (Mesangial cells) from diabetic non-obese diabetic (NOD) mice secrete increased amounts of IGF-1 and this probably contributes to the increased ECM accumulation, largely through an IGF-1 mediated reduction in MMP2 activity.[45]

Hyperglycemia is an important risk factor for the development of diabetic nephropathy. It induces an abnormal activation of protein kinase C (PKC), which is involved in the development of diabetic nephropathy. Upregulation of PKC was observed in kidneys of rat with diabetic nephropathy.[46] It was associated with TGF-β_1, fibronectin, and collagen type IV upregulation. When streptozotocin induced diabetic rats received a PKC inhibitor, LY 333531, there was a down regulation of the above growth factor and ECM proteins. The same inhibitor reduced hyperfiltration and albuminuria in rats and in mice with diabetic nephropathy.[47] The identification of the susceptibility genes in diabetic nephropathy has become the focus of intensive research efforts. Among candidate genes, the PKC-β_1, which encodes β_1 and β_2 isoforms, has been chosen because an abnormal activation of PKC in diabetic patients with nephropathy has been evidenced.[48]

Hyperglycemia is responsible for the presence of high levels of advanced glycosylation end products in patients with diabetes. These glucose metabolites stimulate intrinsic glomerular cells to produce TGF-β_1, which contributes to glomerular sclerosis and tubulointerstitial damage by means of an abnormal ECM production. Forbes et al[49] demonstrated that the administration of ALT 711, an advanced glycosylation end product inhibitor, readily reduced the glomerulosclerosis index, the tubulointerstitial area, and albuminuria in diabetic rats. Moreover, during hyperglycemia, glucose levels rapidly increases in tissues, such as kidney, that are insulin independent for glucose uptake. Excess glucose enters the polyol pathway and activates aldol reductase (AR) but because sorbitol dehydrogenase (SDH) activity does not increase similarly, sorbitol accumulates. To explain the role of the polyol pathway in the onset of diabetic complications, different mechanisms have been proposed: accumulation of sorbitol or fructose, myo-inositol depletion, or alterations in the NADPH / $NADP^+$ and NADH / NAD^+ ratios.[50] In cultured rat mesangial cells, enhanced expression of the facilitative glucose transporter 1 increased AR expression and activity, polyol accumulation and PKC_α levels, which may induce stimulation of matrix protein synthesis.[50] In addition, PKC and polyol pathway activation mediated the high glucose-

induced collagen and fibronectin accumulation by decreasing their degradation.

Hemodynamic dysfunction in patients with diabetes are represented by blood arterial hypertension, glomerular hypertension, and hyperfiltration, Strippoli et al[51] correlated increased plasma endothelin 1 (ET1) levels with the severity of diabetic nephropathy in type 1 and type 2 diabetes. In vitro application of neutralizing ET antibodies indicates ET-1 as a potential risk factor in mesangial proliferation and matrix turnover. A strong argument in favour of ET-1 as a mediator of renal injury derives from the studies with ET receptor antagonists in experimental diabetes. Independent of their blood-pressure-lowering effect, ET receptor antagonists reduced renal ET-1 content, urinary ET-1 excretion and the production of ECM proteins, lowered UAE and reduced renal expression of TGF-β and PDGF.[51] Gnudi et al[52] demonstrated that application of mechanical stretch to mimic a hemodynamic insult induces in vitro GLUT-1 over expression and TGF-β_1 production in rat mesangial cells. The presence of a monoclonal anti TGF-β_1 antibody in vitro-reduced the GLUT-1 expression and the intracellular glucose transport. Mechanical stretch is also responsible for increased glomerular permeability to protein in patients with diabetes. Vascular permeability factor (VPF) is one of the most powerful promoters of this abnormality. Gruden et al[53] studied the effect of stretch on VPF production by human mesangial cells and the intracellular signaling pathways involved. They demonstrated that the application of mechanical stretch for 6 hour induced a 2.4 fold increase over control in the VPF mRNA level. Stretch induced VPF secretion was partially prevented both by PKC inhibitor H7 and by pretreatment with phorbol ester. The combination of both PKC and protein tyrosine kinase (PTK) inhibition completely abolished the VPF response to mechanical stretch[53] and TGF-β_1 and fibronectin production by human mesangial cells. Overall, shear stress is responsible for increased production of growth factors and ECM proteins, which contributes to mesangial cell proliferation and ECM deposition at the glomerular level.

ETHNICITY AND GENETIC RISK FOR MICROALBUMINURIA

Several factors, such as hyperglycemia, hyperlipidemia, hypertension & cardiovascular autonomic dysfunction contribute to the progression of renal damage.[54] However, they are supported by a specific genetic background because only a subset of people with diabetes develops microalbuminuria, irrespective of glycemic control. Evidence supporting this includes the increased risk for microalbuminuria in diabetic siblings of a type 1 diabetic proband with microalbuminuria[55] and familial clustering of nephropathy in both type 1 and type 2 diabetes.[56]

The genetic background was stated many years ago by Klein et al[57] in the Wisconsin epidemiologic study in which they demonstrated that metabolic control did not differ in patients with diabetes, both with and without nephropathy, and a high number of patients with diabetes did not develop the nephropathy despite long term, severe, chronic hyperglycemia. Familial clustering of the disease has been shown by Seaquist et al[55], who reported that siblings of patients with type 1 diabetes and nephropathy have a 4-fold increased risk for developing diabetic nephropathy. The ethnic background plays an important role because some races are more susceptible to diabetic nephropathy than others. In fact, the rate of developing ESRD is five times higher in relatives of black patients with type 2 diabetes in renal replacement therapy.[58] The small tribe of Pima Indians shows a high prevalence of diabetic nephropathy clusters in families with type 2 diabetes. In fact, 14% of descendants of parents with type 2 diabetes without nephropathy develop nephropathy, this percentage is higher in descendants of parents of whom one has proteinuria and increases in descendants of parents of whom both have diabetes and proteinuria.[58]

Diabetic nephropathy is a complex genetic disease in which more genes may be involved in developing the nephropathy. The strategy to search for genes is represented by two different approaches, namely case-control association

Table-3: A Subset of Candidate Genes for Diabetic Nephrophathy

SYMBOL	NAME	POSITION	COMMENT
NHE-1 (SLC9A1)	Na^+/H^+ antiport-1	1p36-1p35	NHE-1 activity is increased in type 1 diabetes with nephropathy
TGF-β (TGF-β1, TGF-β2, TGF-β3)	Transforming growth factor – β (1,2,3)	19q 12-q 13-31, 1q 41, 14q 24	TGF-β mRNA, proteins (and TGF-β receptor mRNA) identified in glomerular cells
GH1	Growth hormone	17q 24	Diabetes causes over expression
IGF-1, IGF-1R	Insulin-like growth factor-1, insulin like growth factor-1 receptor	12q 22-q23, 15q 26.3	
VEGF	Vascular endothelial growth factor	6p 12	VEGF expression increased in glomeruli of diabetic animals; diabetic rats that are treated with anti-VEGF antibody do not have hyperfiltration and reduced AER.
RAAS	ACE, AGT AT1, (AGTR1), AT2 (AGTR2)	17q23, 1q 42-q43, 3q 21-q25, xq22-q23	BP regulation and sodium homeostasis are crucial to progression of nephropathy.

studies and family studies. Candidate gene studies that are based on association have rarely been successful and the familial study approach is not easy because there is no simple Mendelian inheritance model as most affected parents of the patients are dead because there is low life expectancy. For this reason, many family studies are based on analyzing sibling pairs. The National Institutes of Health (USA) established the Family Investigation of Nephropathy and Diabetes Study Consortium to further the linkage analysis studies that led to the mapping of several susceptibility loci for diabetic nephropathy on specific regions of chromosome 3 q for type 1 diabetes and on chromosome 20 and 12 for white siblings pairs with type 2 diabetes.[59]

Many of the studies searching for a specific gene related to microalbuminuria or diabetic nephropathy are limited by insufficient power and failure to define carefully enough the control non-nephropathic groups. Interpretation of the data is further complicated by recent reports that genotype expression varies with the degree of hyperglycemia and with intraglomerular pressure. Opinion is divided as to whether there is one major gene effect or a number of smaller effects. At the moment, no gene with a large effect has been identified. Small effects of a variety of polymorphisms in various genes have been reported, at least in some studies, for example of the renin-angiotensin pathway, peroxisome proliferator activated receptor gamma, endothelial nitric oxide, glucose transporter 1, aldol reductase, and apolipoprotein E.

CONCLUSION

In the last few years, we have witnessed enormous progress in the understanding of the risk factors and mechanisms of diabetic nephropathy, the stages of renal involvement in diabetes, and the treatment strategies to prevent or interrupt the progression of diabetic nephropathy. Tight metabolic and antihypertensive controls remain cornerstone interventions in the treatment. However, diabetic nephropathy, still, remain a huge clinical problem despite implementation of overall intensified glycemic and antihypertensive control in these patients. Extensive research during recent years has identified several new pathways with impact on the development of diabetic kidney disease. It is likely that due to its complexity, targeting multiple points in altered metabolism in the diabetic kidney will be more successful in attenuating the development of diabetic nephropathy, rather than a single approach. Accordingly, there is an ongoing need for development of new therapeutic strategies to prevent the development or retard the

progression of diabetic nephropathy, independent of metabolic control and hypertension. The challenge for future research will be to unravel these complex interactions between hyperglycemia, metabolic factors, hemodynamic factors, intracellular factors, and growth factors/cytokines, which may lead to a better understanding of the pathogenesis of diabetic kidney disease.

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