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DIABETIC NEPHROPATHY – A MAJOR MACROVASCULAR COMPLICATION

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ABSTRACT

Diabetes mellitus (DM) is a complex, progressive disease, which is accompanied by multiple complications. One of the major complication confronted by patients with diabetes is an increased risk of developing diabetic nephropathy (DN) that often progresses to end-stage renal disease.Pathogenesis of DN is multifactorial. The role of hyperglycemia in the pathogenesis of DN has been previously established by a number of studies.Hyperglycemia induces oxidative stress in the rat kidney and increased oxidative stress in the kidney may trigger apoptosis in renal cells in vitro by inducing DNA fragmentation and stimulating expression of apoptosis-regulatory genes. Hyperglycemia also leads to accumulation of advanced glycation end products (AGE's) in renal cortex. These AGE's play a role in the progression of DN through impairment of matrix proteins in vivo, leading to thickening of glomerular basement membrane and expansion of mesangial matrix. DN is also associated with dyslipidemia, which is characterized by higher plasma levels of total cholesterol, low-density lipoprotein and triglycerides, and lower levels of high-density lipoprotein. Reportedly, lipids may induce both glomerular and tubulointerstitial injury through mediators such as cytokines, reactive oxygen species, chemokines, and through hemodynamic changes. A growing body of evidences also suggests that transforming growth factor-β (TGF-β), a fibrogenic cytokine plays a key role in the development of DN. Moreover much advancement has been done to manageDN ascontrol of blood pressure, glucose, and lipids, inhibition of the rennin angiotensin system but these are inadequate to retard the progression of nephropathy.

Keywords: *diabetic nephropathy; type 1 diabetes; type 2 diabetes; hyperglycemia*

INTRODUCTION

Diabetic nephropathy which is damage to kidney, can lead to the chronic renal failure, eventually to death. There are four types of lesions described in diabetic nephropathy; diabetic glomerulosclerosis, vascular lesions, diabetic

pyelonephritis and necrotizing renal papillitis and tubular lesions or Armanni-Ebstein lesion. However, the classical definition of diabetic nephropathy is somewhat different characterized with a progressive rise in urine albumin excretion, coupled with increasing blood pressure, leading to declining glomerular filtration and eventually end stage kidney failure. Recently, links have been found between nephropathy and cardiovascular disease. Diabetic nephropathy have led to the inclusion of premature cardiovascular disease, cardiovascular risk increasing in parallel with albuminuria. Additionally, diabetes has been found the most common reason for progressing to end stage renal disease in the US and in many parts of the world [1]. The number of people getting treatment for end-stage renal failure (ESRF) related to diabetes was 48,374 people in 2008, more than 18-fold what it was in 1980 [2]. Diabetic nephropathy is now the single commonest cause of ESRF worldwide and is acknowledged as an independent risk factor for cardiovascular disease. Today, Diabetic renal disease describes not only diabetic nephropathy but also atheroembolic disease, ischemic nephropathy, and interstitial fibrosis that occurs as a direct result of diabetes [3], [4]. The number of people initiating treatment for ESRF related to diabetes was 48,374 people in 2008, more than 18-fold what it was in 1980 [2]. In many countries, including the Middle East the majority of diabetic patients starting kidney replacement therapy now have type 2 rather than type 1diabetes [5]. This review will therefore discuss nephropathy in both type 1 and type 2 diabetes.

Diabetes mellitus

The term diabetes mellitus (DM) encompasses metabolic disorders of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs. DM may lead to characteristic symptoms such as thirst, polyuria, blurring of vision and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and in absence of effective treatment, even death. Often symptoms are not severe or may be absent and consequently hyperglycemia, sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The chronic effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints and features of autonomic dysfunction, including sexual dysfunction. Data has shown that people with diabetes are at great risk of cardiovascular, peripheral vascular and cerebrovascular disease [6].

The term 'diabetes' was coined by Aretaeus of Cappadocia. The Greek word *diabaínein*literally means "passing through" or "siphon," a reference to one of diabetes' major symptoms-excessive urine production. The word became "diabetes" from the English adoption of the medieval Latin *diabetes*. In 1675 Thomas Willis added *mellitus* from the Latin word for honey (*mel*in the sense of "honey sweet") when he observed that the blood and urine of a diabetic patient has a sweet taste. This was noticed long before in ancient times by the Greeks, Chinese, Egyptians and Indians. In 1776, it was assured that the sweet taste was because of an excess of a kind of sugar in the urine and blood of people with diabetes.

Classification

Although all forms of diabetes mellitus share hyperglycemia as a common feature, the pathogenic processes involved in the development of hyperglycemia vary widely. The previous classification of diabetes mellitus were based on the age at onset of disease or on the made of therapy; in contrast, the recently revised classification reflects our greater understanding of the pathogenesis of each variant [7]. The etiologic classification of diabetes mellitus is given in table 1.

Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)

Type 1 Diabetes was previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes. It is characterized as pancreatic beta cells destruction by the body's immune system, the only cells in the body that make the hormone insulin that regulates blood glucose. This form of diabetes usually strikes children and young adults, although disease onset can occur at any age. Type 1 diabetes may account for 5% to 10% of all diagnosed cases of diabetes. Among various risk factors main risk factors for type 1 diabetes may include autoimmune, genetic and environmental factors [7].

Type 1 DM is a chronic autoimmune disease associated with selective destruction of insulin producing pancreatic β-cells. The onset of clinical disease represents the end stage of β-cells destruction leading to type 1 DM. Several features characterize type 1 DM as an autoimmune disease [8].

1. Presence of immuno-competent and accessory cells in infiltrated pancreatic islets.
2. Association of susceptibility to disease with the class II (immune response) genes of the major histocompatibility complex (MHC), human leucocyte antigens (HLA).
3. Presence of islets cell specific auto-antibodies.
4. Alteration of T cell mediated immunoregulation, particularly in CD4+ T cell compartment.
5. Response to immunotherapy.

Idiopathic diabetes

Researches have shown that there is no well known etiology for some forms of type-1 diabetes. Many of these patients have permanent insulinopenia and are prone to ketoacidosis, without having any evidence of autoimmunity [7],[9],[10]. Although only a minority of patients with type-1 diabetes fall into this category, of those who do, most are of African or Asian ancestry. Main characteristic of the individuals with this form of diabetes are episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly associated genetically, lacks immunological evidence for β-cell autoimmunity, and is not HLA associated. An absolute requirement for insulin replacement therapy in affected patients may required.

Type-2 diabetes (non-insulin dependent)

Type-2 diabetes previously also named non-insulin-dependent diabetes mellitus (NIDDM or adult-onset diabetes) more prevalent in the Western world occurs in approximately 90–95% of people resulting from insulin resistance and insufficient compensatory insulin secretion. The disease has an insidious onset and remains asymptomatic and undiagnosed for a long period, however the presence of moderate hyperglycemia is able to induce severe diabetic late complications [11],[12],[13]. Type-2 diabetes has familial history and is strongly favored by genetic predisposition. However, although it shows familial aggregation as well as a high concordance (80%) in monozygotic twins, its mode of inheritance is not fully understood. It may well be a polygenic disease. In any case, the risk of offspring and siblings of type-2 diabetic patients to develop the disease is relatively elevated.

In addition to the genetic predisposition, environmental factors also influence onset of type 2 diabetes, such as excessive caloric intake, obesity with increased body fat in the abdominal (visceral) site, sedentary habit, etc [14]. The insulin levels may be normal or even increased (especially in presence of obesity) for a long time, but may decrease in the late stage of the disease. The abnormal hyperglycemia condition can be early identified measuring fasting glycemia (FPG) or performing an oral glucose tolerance test (OGTT). This type of diabetes is non-insulin-dependent for survival and is non-ketosis prone [12],[15] . Hyperglycemia is usually improved or corrected by diet, weight loss and oral hypoglycemic drugs. In type-2 diabetics short-term life-threatening complication, the nonketotic hyperosmolar coma, can develop whereas ketoacidosis seldom occurs spontaneously, although it may arise during stress, infections or other illnesses.

1-***Diabetic Nephropathy- a major diabetic complication***

DN is one of the most severe complications of DM and has become the largest cause of end-stage renal disease [16]. It affects more than one third of patients with type 1 DM, and up to 25% of all patients with type 2 DM; thus profoundly contributing to patient morbidity and mortality [17],[1],[18]. Nearly 30% of chronic renal failures in India are due to DN [19]. Nonetheless, attention towards DN is not directed until the patient has progressed towards the stage of renal failure.

Epidemiology of diabetic nephropathy

The prognostic value of a small amount of albumin in urine for the development of kidney damage in patients with type 1 or 2 DM was confirmed in the early 1980's. This stage of kidney damage was called the microalbuminuria stage or initial nephropathy [20]. Approximately 20-30% of the patients develop microalbuminuria after 15 years of disease duration and less than half develop real nephropathy [21]. The European Diabetes(EURODIAB) Prospective Complications Study Group and 18-year Danish study by Chaturvedi et al.[22] showed that the overall occurrence of microalbuminuria in patients with type 1 and 2 DM is12.6% (after 7.3 years) and 33%, respectively. According to the United Kingdom Prospective Diabetes Study (UKPDS), the annual incidence of microalbuminuria in patients with type 2DM in Great Britain is 2% and the prevalence is 25% ten years after the diagnosis. Proteinuria develops in approximately 15-40% patients with type 1 DM, usually after 15-20years of DM duration [23]. In patients with type 2 DM, the prevalence varies between 5%and 20% on average [24]. Diabetic nephropathy is more frequent in African Americans, Asian Americans, and Native Americans [25]. The occurrence of diabetic nephropathy in Pima Indians is very interesting, indeed. According to a study published in 1990, around 50% of Pima Indians with type 2DM developed nephropathy after 20 years of the disease, and 15% of them were already in the terminal stage of kidney failure [26].

Diabetes is responsible for 30-40% of all end-stage renal disease (ESRD) cases in the United States [27]. In terms of diabetic kidney disease in the United States, the prevalence increased from 1988-2008 in proportion to the prevalence of diabetes [28]. Racial differences in the prevalence of DN have been reported. Some studies conducted in the U.K. and Europe has reported increased prevalence of DN in migrant Asian Indians compared with white Caucasians [25],[29]. Migrant Asian Indians had 40 times greater risk of developing ESRD when compared with the Caucasians [30]. The prevalence of nephropathy in India was less when compared with the prevalence in Asian Indians in the UK in the study by Samanta et al [31]. The prevalence of DN in type 2 diabetic subjects is reported to be 5-9% from various Indian studies [32],[33],[34] The combination of hypertension and diabetes is an especially dangerous clinical situation, both for risk of microvascular and macrovascular complications of diabetes and for diabetes-related and overall mortality. Hypertension is unfortunately also very common in patients with diabetes.

Hypertension occurs in 50% of patients with diabetes and results in a seven fold increase in mortality. Concomitant nephropathy in patients with diabetes and hypertension results in a 37-fold increase in mortality. Important clinical concomitants of diabetic nephropathy are retinopathy and cardiovascular disease. Nearly all patients with diabetic nephropathy will also have developed retinopathy. This has important implications for screening and attention to preventive measures in the care of patients with diabetes. The reverse is not as frequently true. That is, a much lower percentage of patients with retinopathy will have evidence of renal involvement [12],[35].

Diabetic renal disease is also closely associated with coronary artery disease. The presence of microalbuminuria is a powerful predictor of death from cardiovascular disease. Thus, the presence of diabetes, especially if accompanied by microalbuminuria, is a signal for very aggressive attention to all cardiovascular disease risk factors and for improvement to the fullest extent possible by lifestyle modification and pharmacotherapy when appropriate. In addition to direct microvascular effects of diabetes on the heart, associated mechanisms may include hyperlipidemia, hypertension, and coagulation abnormalities [36].

1. Signs and symptoms of diabetic nephropathy

The clinical course of DN includes an initial increase in glomerular filtration rate, thickening of the glomerular basement membrane, expansion of the mesangium, microalbuminuria, proteinuria, and eventually a decline in glomerular filtration [37]. As renal function declines, arterial blood pressure is increased. Systemic hypertension further contributes to the rate of progression to nephropathy and eventually the syndrome can progress to ESRD [38]. Throughout its early course, DN has no symptoms. They develop in late stages which is shown in table 2.

The first laboratory abnormality is a positive microalbuminuria test. Most often, the diagnosis is suspected when a routine urinalysis of a person with DN shows too much protein in the urine (proteinuria). The urinalysis may also show glucose in the urine, especially if blood glucose is poorly controlled. Serum creatinine and blood urea nitrogen may increase as kidney damage progresses. A kidney biopsy confirms the diagnosis, although it is not always necessary if the case is straightforward, with a documented progression of proteinuria over time and presence of diabetic retinopathy on examination of the retina of the eyes [39],[40],[41].

2. Pathophysiology of diabetic nephropathy

Pathophysiology deals with the study of the characteristics, causes, and effects of disease, as observed in the structure and function of the body, especially changes in body tissues and organs that cause or are caused by disease. In recent years, the knowledge of the pathophysiologic processes leading to DN has notably improved on a genetic and molecular level. The classical view of renal injury as consequence of metabolic and hemodynamic alterations has been transformed significantly, with clear evidence indicating that these traditional factors are only partial aspects of a much more complex scenario [4],[42],[43].

Renal lesions in type 1 and type 2 DM

The renal lesions underlying renal dysfunction differ in type 1 and type 2 DM, although the clinical manifestations of DN which includes proteinuria, decreased glomerular filtration rate and increasing blood pressure are similar. In type 1 DM, the most important structural changes involve the glomerulus predominantly, whereas light microscopy studies have shown that a substantial proportion of type 2 diabetic patients have more advanced tubulo-interstitial and vascular than glomerular lesions [44],[45].

In type 1 DM, glomerulopathy is the most important lesion, characterized by mesangial expansion, glomerular basement membrane (GBM) thickening and glomerular sclerosis [46]. GBM thickening, the first measurable change, has been documented as early as 1.5 to 2.5 years after the onset of type 1 DM [47],[48]. Mesangial expansion develops later; an increase in the matrix component of the mesangium can be detected as early as 5-7 years after the onset of DM [49],[50]. Diffuse and generalized mesangial expansion, commonly termed diffuse diabetic glomerulosclerosis, can be associated with nodular lesions consisting of areas of marked mesangial expansion forming large round fibrillarmesangial zones with palisading of mesangial nuclei around the periphery of the nodule and extreme compression of the associated glomerular capillaries (Kimmelstiel-Wilson nodules). Both mesangial expansion and GBM thickening are consequence of extracellular matrix (ECM) accumulation, with increased deposition of types IVand VI collagen, laminin and fibronectin[51],[52].

Additional structural abnormalities in type 1 DM include glomerular enlargement, tubular basement membrane (TBM) thickening, tubular atrophy [53], interstitial expansion [54], afferent and efferent arteriolar hyalinosis [55]. Afferent and efferent arteriolar hyalinosis occurs few years after the onset of DM. This is an exudative lesion, mainly due to the replacement of the smooth muscle cells by plasma proteins, especially immunoglobulins, complement, fibrinogen and albumin. The severity of arteriolar hyalinosis is significantly correlated with percent

sclerosed glomeruli, suggesting that this vascular lesion could contribute to global glomerular sclerosis through severe compromise of glomerular blood flow. Also, Bowman's capsule thickening is regularly present.

In Danish type 2 diabetic patients with proteinuria, Osterby et al. [56] described great variability in glomerular injury; they outlined that type 2 diabetic patients tended to have less marked glomerular changes than type 1 with comparable renal function. Similar to this finding, Fioretto et al. [57] reported that glomerular lesions were less advanced in type 2 than type 1 diabetic patients and a substantial number of these patients had normal glomerular structure despite abnormal albumin excretion rate. Brocco et al. [45] studied fifty-three type 2 diabetic patients with microalbuminuria in greater detail and proposed a classification system including 3 major groups:

Category CI: Normal or near-normal renal structure.
These patients (41 %) had biopsies which were normal or showed mild mesangial expansion, tubulo-interstitial changes, or arteriolar hyalinosis.
Category CII: Typical diabetic nephropathology.
These patients (26 %) had established diabetic lesions with an approximately balanced severity of glomerular, tubulo-interstitial and arteriolar changes. This picture was typical of that seen in type 1 diabetic patients with obvious light microscopy DN changes.
Category CIII: Atypical patterns of renal injury.
These patients (33 %) had relatively mild glomerular diabetic changes despite disproportionately severe renal structural changes: (a) tubular atrophy, tubular basement membrane thickening and reduplication and interstitial fibrosis (tubulo-interstitial lesions); (b) advanced glomerular arteriolar hyalinosis commonly associated with atherosclerosis of larger vessels; and (c) global glomerular sclerosis. In the CIII group, these patterns were present in all possible combinations. No cases of definable non-diabetic renal disease were found in this series of 53 patients [58],[59],[60].

Relationship between structural abnormalities in kidney and renal function in type 1 and type 2 DM
Mesangial expansion and interstitial expansion are independent determinants of renal dysfunction in DM and are probably consequence of different pathogenetic mechanisms. The clinical manifestations of DN are strongly related with the structural changes, especially with the degree of mesangial expansion in both type 1 and type 2 DM [57].

Mesangial expansion, morphometrically termed as mesangial fractional volume [Vv(mes/glom)] (the percentage of the cross-sectional area of the glomerular tuft made up by mesangium) is the structural parameter that best correlates with all functional parameters in type 1 DM. [61],[62]. Indeed, a highly significant inverse correlation exists between Vv (mes/glom) and glomerular filtration rate (GFR); when mesangium expands it restricts and distorts glomerular capillaries and diminishes capillary filtration surface, which is strongly inversely related to Vv(mes/glom) and directly to GFR [63]. Vv (mes/glom) is also related to albumin excretion rate (AER) and blood pressure [64]. In contrast, GBM thickening is not related to GFR or to the presence of hypertension, but only to AER, suggesting that this lesion is involved in the pathogenesis of albuminuria, rather than in the loss of kidney function. Also, interstitial expansion and percentage of global sclerosis are related to proteinuria, hypertension and declining GFR [54],[55]. In the early stages of DN, progression from normoalbuminuria to microalbuminuria and from microalbuminuria to early overt nephropathy is related only to progressive mesangial expansion without any progression in interstitial fibrosis or GBM thickening [65]. These data are partially in contrast with a recent study describing that GBM width at baseline biopsy was predictive of AER after six years of follow-up [66].

In Japanese type 2 patients with a wide range of renal function, morphometric determinations of diabetic glomerulopathy showed correlations with renal functional parameters similar to those observed in type 1 DM [67]. However, more recent studies suggest a high incidence of normal glomerular structure among microalbuminuric and proteinuric Japanese type 2 diabetic patients [68].
Study on a large group of Caucasian type 2 diabetic patients revealed that diabetic glomerulopathy was less apparent in patients with type 2 DM than in those with type 1 DM and had similar renal function [69]. AER was directly related to both GBM width and Vv (mes/glom), whereas GFR was inversely related to Vv(mes/glom) but not to GBM width. Although significant, these structural/functional relationships were less precise than in type 1 DM. In a recent longitudinal study, [70] considered the predictive value of glomerular structure in Pima Indians with type 2 DM and microalbuminuria relative to the change in urinary albumin excretion during 4-year follow-up. They found that the number of podocytes per glomerulus was the strongest predictor of changes in albuminuria, and that fewer cells were predictive of more rapid progression during follow-up.

6. Risk factors for pathogenesis of diabetic nephropathy
The pathogenesis of DN is a multifactorial process. Two major causative factors have been implicated in the development of DN: metabolic and hemodynamic. Studies in type 2 DM patients suggest that, although poor metabolic control is the most important determinant of the development of nephropathy, hypertension and

hyperlipidemia are also involved [71]. Genetic susceptibility is yet another factor that has been proposed to play an important role in the development and progression of DN. The major factors are as follows:

a) Hyperglycemia

There is no doubt that poor glycemic control is associated with DN. Hemoglobin A1C (HbA1C) levels are higher in patients with micro- and macroalbuminuria than in those with normoalbuminuria [72]; and in two longitudinal studies the glycemic control predicts the future development of microalbuminuria in normotensive type 1 diabetic patients with normoalbuminuria [73],[74]

Intervention studies have demonstrated the renoprotective effect of an optimum glycemic control in both human and experimental DN. In animals, structural glomerular changes and albuminuria can be prevented by the maintenance of normoglycemia by either islet cell transplantation or intensive insulin therapy or reversed by transplantation of an affected kidney into a nondiabetic animal [75,[76],[77]. In humans, the Diabetes Control and Complications Trial (DCCT), a prospective multicenter randomized clinical trial, comparing in 1,441 type 1 diabetic patients the effect of intensive and conventional insulin therapy for the risk for development and progression of diabetic chronic complications, has demonstrated that a sustained improvement of HbA1C reduces the risk for development of DN [78]. A Japanese randomized study similar in design to the DCCT involved 110 type 2 diabetic patients followed for up to 6 years. The risk of developing nephropathy in the well-controlled group (HbA1c 7.1%) was 70% lower compared to that of poorly controlled patients (HbA1c 9.4%) [79]. Similarly, the U.K. Prospective Diabetes Study has shown that improved glycemic control is effective in the prevention of microalbuminuria in newly diagnosed type 2 diabetic patients [80]. Finally, in a small study in eight type 1 diabetic patients, pancreas transplantation and near normoglycemia for 10 years reversed kidney structural abnormalities [81].

b) Hypertension

There is evidence that hypertension plays a critical role in the progression of DN. Indeed, the development of proteinuria is paralleled in most cases by a gradual increase in systemic blood pressure, and there is a significant correlation between the blood pressure levels and the rate of decline in glomerular filtration rate [82]. Furthermore, intervention studies in both animals and humans have demonstrated significant renoprotective and antiproteinuric effects of antihypertensive therapy [83],[84]. In DN, hypertension is not merely the result of relentless kidney damage. There is considerable clinical evidence that the elevated arterial pressure is also important in the genesis of the glomerular lesion. In Pima Indians, higher mean blood pressure before the onset of DM actually predicts an abnormal albumin excretion rate after the diagnosis of DN [85] Prospective studies in patients with both type 1 and type 2 DM and normal albumin excretion have demonstrated that mean arterial pressure levels are significantly higher in those patients who progress to microalbuminuria than those who do not progress [86],[74]. Systemic hypertension contributes to the development of DN via associated glomerular hypertension. Under normal conditions, intraglomerular capillary pressure is tightly regulated by precise adjustments in afferent and efferent arteriolar resistance. Hyperglycemia induces vasodilatation, and in DN there is a marked reduction in afferent and a lesser reduction in efferent arteriolar resistance. This leads to an increase in glomerular capillary pressure levels and allows ready transmission of any increase in systemic blood pressure to the glomerular capillary network [87]. Hemodynamic factors alter the function of glomerular, mesangial, and epithelial cells, which results in an increase in mesangial matrix formation and basement membrane thickening [37]. Vasoregulatory peptides such as endothelial-derived relaxing factor, tissue plasminogen activator, endothelin- 1, and platelet-derived growth factor beta are also affected by intraglomerular hemodynamic factors. An increase in systemic blood pressure ultimately leads to extracellular matrix accumulation, increased glomerular permeability, proteinuria, and glomerulosclerosis [37].

c) Dyslipidemia

Dyslipidemia is common in patients with DN and is considered as a risk factor for the progression of DN [88],[71]. Diabetic patients often have multiple lipoprotein abnormalities such as, increased plasma levels of very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and triglycerides [89]. In addition to the abnormalities in amount of lipoprotein, the diameter of LDL particles is also reported to be smaller in patients with DN [90] compared to diabetic patients without nephropathy. Experimental studies in animal models demonstrate that lipid abnormalities contribute to glomerulosclerosis [91]. Ravid et al. [92] found that the concentration of cholesterol, both initially and during a five year follow up period, was positively related with the subsequent increase in urinary albumin excretion in microalbuminuric patients with non-insulin dependent DM. Dominguez et al.[93] also found a direct linkage between renal injuries of rats with type 2 DM and elevated levels of blood LDL cholesterol. Recent study demonstrating that hyperlipidemia and hyperglycemia act synergistically to induce renal injury in LDL receptor-deficient BALB mice. Spencer et al. [94] has further indicated that lipid can exacerbate DN.

d) Genetic factor

Several genes seem to contribute to the susceptibility to DN. However, the distinct susceptibility genes have not yet been identified [17]. A susceptibility locus for DN on chromosome 3q has been found in a study that included 66 US Caucasian discordant sibling pairs [95]. The locus spanned a 20 cM region around the angiotensin II type 1 receptor

(AT1) gene. Two polymorphic microsatellite markers (D3S1308 and a (CA)*n* dinucleotide repeat polymorphism at the 3' flanking region of the AT1 gene) located in the vicinity of this gene showed a maximum strength of linkage, while single nucleotide polymorphisms (SNPs) within the gene itself had no association with DN [95]. The findings of Chistiakov et al.[96] suggest that the DN susceptibility locus, which is responsible for the associations with clinical nephropathy in Russian diabetic patients, seems to be distinct from the AT1 gene in agreement with Moczulski et al [97]. At present the genomic region in which markers D3S1512, D3S1550, D3S2326, and D3S3599 (that showed an association with DN) are located, has no known or characterized genes. However, there are two genes (ATP1B3 and CHST2) located relatively close (0.5-1.1Mb centromeric) to this region which should be functionally relevant to be a candidate for the susceptibility to DN.

Interestingly, for DN in type 2 DM, a genome-wide scan also found a susceptibility locus on chromosome 3q in Pima Indians [98]. The peak multipoint LOD score of 1.48 was obtained for marker D3S3053 [98] which lies about 23Mb telomeric to the (CA)*n*-AT1 polymorphic microsatellite, a peak marker for linkage to DN in type 1 DM [95]. This distance is quite large to represent distinct susceptibility loci for DN in type 1 and type 2 DM. Fine mapping with additional markers in this region should be helpful to clarify whether they represent the same loci or different loci [99].

e) Other factors

Other factors that are associated with DN include age, male sex, presence of retinopathy and cigarette smoking [88]. There tends to be a more rapid progression to nephropathy in smokers compared with nonsmokers. Cessation of smoking alone may reduce the risk of progression by 30% in patients with type 2 DM [100]. Gall et al.[88], in a prospective observational study involving 176 patients with type 2 DM, found that males had a 2.6 times greater risk of developing incipient or overt nephropathy. He also found that increasing age was significantly associated with abnormally increased urinary albumin excretion rate in both univariate and multivariate analysis. However, Klein et al. [101] found that younger age at diagnosis was significantly associated with a decrease in the estimated annual creatinine clearance in patients with type 1 DM. A close relation between the presence of diabetic retinopathy and risk of developing an abnormally high urinary albumin excretion rate has also been reported [102].

7. Mechanisms involved in diabetic nephropathy

a) Immunologic mechanisms in diabetic nephropathy

Traditionally, DN has been considered a non-immune disease. However, recent studies have shown that long-term, innate immune system activation resulting in chronic inflammation is a part of the insulin resistance syndrome and is associated with the risk of developing type 2 DM, implying that immune-mediated inflammatory processes may play a significant role in the pathophysiology of DM and its complications [103],[104]. Leukocytes, monocytes, and macrophages [105],[106], as well as different molecules, such as chemokines [107], adhesion molecules [108], growth factors [109], nuclear factors [110],[111], and cytokines [112] have been implicated in diverse pathogenic pathways related to DN.

A potential participation of inflammatory cytokines in the pathogenesis of DN was suggested for the first time in 1991. In that year, Hasegawa et al [113] demonstrated that peritoneal macrophages cultured with glomerular basement membranes from diabetic rats produced significantly higher amounts of the inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) than macrophages cultured with glomerular basement membranes from normal rats. After that initial study, other experimental works have demonstrated that in the kidney, both blood-borne cells (mainly monocytes and macrophages), as well as diverse intrinsic renal cells (endothelial, mesangial, dendritic, tubular epithelial cells), are able to synthesize inflammatory cytokines [114],[115]. Distinct from their role as mediators of immunological reactions and inflammatory processes, inflammatory cytokines have been associated with significant renal effects which play a significant role in the development of renal injury in type 2 DM. These renal effects of inflammatory cytokines with relevance for DN are listed as below [116],[117],[118],[112].

b) Uncoupling of VEGF with NO as a mechanism for diabetic nephropathy

Vascular endothelial growth factor (VEGF), a potent factor for new vessel formation [119] exhibits beneficial effects in both acute and chronic non-diabetic renal disease [120],[121]. On the other hand, VEGF exhibits deleterious effects in DM by mediating renal hypertrophy, an increase in glomerular filtration rate and increased urinary protein excretion [122],[123]. A potential explanation for these paradoxical effects of VEGF could be related to reduced bioavailability of endothelial nitric oxide (NO) in subjects with DN. VEGF stimulates endothelial NO production and this mechanism has a beneficial role to maintain endothelial cell viability, inhibit vascular inflammation and suppress the vascular smooth muscle cell activation [119] resulting in maintenance of vascular integrity. However, it has been known that NO bioavailability is reduced in DM [124], while VEGF expression is high in the diabetic kidney due to the effects of hyperglycemia [125]. Hence, the reduced NO bioavailability could theoretically result in an "uncoupling of VEGF with NO". Interestingly, there is evidence that blockade of endothelial NO results in a compensatory increase in VEGF [126], which engages an "NO-independent pathway" to

stimulate an excessive proliferation of endothelial cells [127]. This may account for the abnormal angiogenesis in human and rodent DN. This hypothesis is supported by several in vivo studies demonstrating that a long term inhibition of NO synthase (NOS) resulted in severe vascular disease along with de novo VEGF expression in renal vessels as well as in the coronary artery, in which endothelial proliferation, monocyte infiltration and vascular smooth muscle cell proliferation were prominent [121]

c) Signaling Mechanisms involved in diabetic nephropathy

There is strong evidence that hyperglycemia is necessary in the pathogenesis of DN, and some of the mechanisms that link hyperglycemia to the functional/structural abnormalities of diabetic kidney disease have been elucidated. Extracellularly, glucose reacts nonenzymatically with primary amines of proteins, forming glycated compounds like amadori products. These glycated proteins undergo progressive dehydration, cyclization, and rearrangement to form advanced glycation end products (AGE) [128]. AGE can interfere with protein function and promote formation of aggregates. In kidney, AGE can become trapped in glomerular basement membranes and covalently crosslink to collagen resulting in membrane thickening and distortion [129]. In addition to these direct effects, AGE can bind to AGE-specific receptors present on many cell types, including mesangial cells [130]. Interaction of AGE-modified proteins with the AGE receptors serves to degrade AGE proteins, but also induces the synthesis and release of cytokines, such as transforming growth factor-β1 (TGF-β1), connective tissue growth factor (CTGF), platelet-derived growth factor, vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP- 1), and insulin-like growth factor (IGF), and results in enhanced collagen, laminin, and fibronectin production [131],[132].

In diabetic rats both renin and angiotensinogen gene expression are increased in the kidney, and the intensity of ACE immunostaining is enhanced in both glomeruli and renal vessels [133]. The angiotensin II receptor is also overexpressed in early experimental DN, suggesting increased local responsiveness to angiotensin II [134]. In both mesangial and tubular epithelial cells, angiotensin II directly induces matrix deposition via a TGF-β1-dependent mechanism and stimulates production of MCP-1, a potent monocyte chemoattractant[135]. In glomerular epithelial cells angiotensin II induces apoptosis via a TGF-β1-dependent mechanism [136]. In isolated glomeruli, angiotensin II increases glomerular permeability to protein and impairs the size-selective function of the glomerular filtration [137].

Enhanced polyol pathway activity has been demonstrated in diabetic glomeruli in humans [138]. However, the initial hypothesis that sorbitol accumulation and myoinositol reduction cause tissue damage is unlikely to operate in the kidney, because compensatory mechanisms prevent inositol depletion in kidney cells [139]. In the polyol pathway, excess sorbitol is oxidated to fructose by the enzyme fructose dehydrogenase, a process that increases the ratio of NADH/NAD and may result in cellular oxidative stress. In addition, fructose is a reactive sugar that can lead to AGE production [140]. A series of studies of aldose reductase inhibitors (ARIs) have produced inconclusive results in both experimental and human DN. More recently, treatment of diabetic rats for 6 months with the ARI tolrestat resulted in a slight reduction in albumin excretion rate [141], but to date no convincing effect of aldose reductase inhibitors has been reported in controlled studies in humans. This indicates that the polyol pathway activation is more likely to be an epiphenomenon and that other more central mechanisms are operating in the pathogenesis of DN.

There is increasing evidence that the overproduction of reactive oxygen species (ROS) is one major factor in the development of DN. The overproduction of ROS is a direct consequence of hyperglycemia [142]. In addition to their ability to directly inflict damage to DNA, protein, lipid, and carbohydrate, ROS can function as signaling molecules to activate a number of cellular stress-sensitive pathways that cause cellular damage [143]. ROS mediate hyperglycemia-induced activation of signal transduction cascades and transcription factors leading to transcriptional activation of profibrotic genes. Protein kinase C (PKC), transforming growth factor-b1 (TGF-b1) and angiotensin II (Ang II) stimulated by hyperglycemia-induced ROS, in turn, generate and signal through ROS and thus ROS act as a signal amplifier in DN [144]. Numerous studies have explored the role of TGF-β1 in diabetic glomerulosclerosis, and on the basis of several evidences, it is now generally believed that TGF-β1 is the mediator of a final common pathway leading to sclerosis in DM. In both human and experimental DM, TGF-β1 gene expression and protein secretion are increased in the glomeruli and in the tubuli [145]. In diabetic mice TGF-β blockade significantly reduces both type IV collagen and fibronectin overexpression and prevents glomerular hypertrophy, glomerulosclerosis, and renal insufficiency [146],[147]. Mesangial cell exposure to high glucose also induces TGF-β receptor overexpression, suggesting that high glucose may also enhance the response to TGF-β1 [148]. On the other hand, TGF- β1 induces the GLUT-1 transporter in mesangial cells and can thereby enhance glucotoxicity [149] Increased flux through the hexosamine biosynthetic pathway also enhances both TGF-β1 expression and activity in mesangial cells in vitro [150]. The link between hyperglycemia, TGF-β1, and kidney sclerosis is thus well established.

8. ***Recent advances in pharmacotherapy for diabetic nephropathy***

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, and possibly control of hyperlipidemia and restriction of

protein intake. On the other hand, angiotensin converting enzyme inhibitor (ACE-I) and angiotensin receptor antagonist (ARB) have been reported to reduce the progression of DN to some extent [151]. These treatments are effective in the early stage of DN but are hardly effective in the advanced stage when there is an increase in serum creatinine. Patients who develop end stage renal disease ESRD will require renal replacement therapy.

a) Glycemic Control

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 [78],[79]. Furthermore, in a small study in eight type 1 diabetic patients, pancreas transplantation and near normoglycemia for 10 years reversed kidney structural abnormalities [81]. The American Diabetes Association [152] recommends that the therapy should focus on obtaining target pre-prandial glucose of 80-120 mg/dL (whole blood) or 90-130 mg/dL (plasma); bedtime glucose of 100-140 mg/dL (whole blood) or 110-150 mg/dL (plasma), and HbA1c of <7%. Target blood sugar levels can be achieved using oral hypoglycemic agents, insulin, or a combination of both.

b) Blood pressure control

Antihypertensive therapy has been shown to be beneficial in both type 1 and type 2 diabetic patients with nephropathy. Blood pressure goals have been set by both the ADA (American Diabetes Association [152], and the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure (The Sixth report, 1997). For diabetic patients in general, the target blood pressure is >130/80 mmHg. The target is even more stringent, >120/75 mmHg, for patients who have >1 g proteinuria. Blockade of the renin-angiotensin system with either ACE inhibition or angiotensin II receptor blockade is the first line of therapy in type 1 and type 2 respectively[153],[154]. There is even evidence that use of an ACE inhibitor in nondiabetic subjects may lower the risk of developing DN by some 30% (Heart Outcomes Prevention Evaluation (HOPE) Study Investigators, 2000) and this is also true of the angiotensin II receptor- blocking agent losartan (Cozaar) when compared to atenolol [155]. There is, however, a theoretical benefit and indeed some clinical evidence that combining ACE inhibitors and angiotensin II receptor blockers may be more effective than using either agent alone in reducing both proteinuria and blood pressure [156].Other agents that can be used to lower blood pressure include β blockers [157], nondihydropyridine calcium channel blockers [158], dihydropyridine calcium channel blockers [159] and diuretics like thiazides [160]. In an analysis of five recent clinical hypertension trials, a combination of three antihypertensive medications on average was required to reach goal blood pressure [161]. Some patients may need five or more medications with different antihypertensive mechanisms of action to achieve adequate control.

c) Control of Hyperlipidemia

There is suggestion that elevation in lipid levels may contribute to the development of glomerulosclerosis. Studies have shown that lipid lowering may have a beneficial effect on renal function [162]. A meta-analysis of 13 controlled trials involving a total of 362 subjects, 253 of whom had DN, showed that statins decreased proteinuria and preserved GFR in patients with chronic renal disease [163]. These effects could not be entirely explained by a reduction in blood cholesterol. 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 DM. Primary therapy should focus on obtaining LDL levels of l00 mg/dL, triglyceride levels <l50 mg/dL, and HDL levels >45 mg/dL for men and >55 mg/dL for women [152].

d) Protein Restriction

The role of dietary protein restriction in chronic renal disease is controversial [164],[165] However, restriction of protein (0.6 g of protein/kg body weight per day) and phosphorus (500 mg to 1 g of phosphorus per day) was shown to reduce the decline in glomerular filtration rate, lower blood pressure, and stabilize renal function compared with a higher intake of protein and phosphorus in a randomized trial involving patients with type 1 DM and overt nephropathy [166]. In addition, restriction of protein intake to 0.8 g/kg body weight per day, which is consistent with the recommended daily allowance, has been shown to reduce the rate of progression to ESRD in patients with type 1 DM in another study [167]. The National Kidney Foundation recommends that patients with GFR <29 mL/min per 1.73m2 should have a daily protein intake of 0.6 g/kg body weight [168].

e) Multifactorial Treatment

Experimental and clinical studies have shown that the optimal therapeutic approach in the treatment of DN may be intensive combined therapy targeting hyperglycemia, hypertension, microalbuminuria, and dyslipidemia [169],[170]. The Steno type 2 Study compared an intensive multifactorial intervention to standard therapy in 160 patients with type 2 DM [171]. There was 73% reduction in the incidence of clinical proteinuria in the multifactorial intervention group. In addition, the intensive therapy was also more effective in lowering HbA1c values (7.6% vs. 9.0%), fasting plasma glucose (134 vs. 185 mg/dL, LDL cholesterol (112 vs. 127 mg /dL [2.9 vs. 3.3 mmol /L]), systolic BP (138 vs. 145 mmHg) and the rate of progression of retinopathy and autonomic neuropathy.

Conclusions

In the last several years, we have witnessed an enormous progress made not only in our understanding of the risk factors and mechanism of the development of diabetic nephropathy, but DN is currently the major indication for

kidney replacement therapy worldwide, and patients with diabetes developing end-stage kidney disease likely to increase. Early detection of DN along with the treatment of main risk factors (hyperglycemia, hypertension, and dyslipidemia) and use of inhibitory drugs mechanism (ACEI and ARB) may decrease the progression of the disease. The treatment of increased blood pressure is a priority. All listed measures also lead to a decrease in the overall and cardiovascular mortality in patients with DM. Novel therapies are in advanced stages of development, but the current challenge is to develop ways of better applying those that we already know to be effective [172].

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Table 1—Etiologic classification of diabetes mellitus

I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)
A. Immune mediated
B. Idiopathic
i) II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)

III. Other specific types

A. Genetic defects of β-cell function

1. Chromosome 12, HNF-1α (MODY3)

2. Chromosome 7, glucokinase (MODY2)

3. Chromosome 20, HNF-4α (MODY1)

4. Chromosome 13, insulin promoter factor-1 (IPF-1; MODY4)

5. Chromosome 17, HNF-1β (MODY5)

6. Chromosome 2, *NeuroD1* (MODY6)

7. Mitochondrial DNA

8. Others

B. Genetic defects in insulin action

1. Type A insulin resistance

2. Leprechaunism

3. Rabson-Mendenhall syndrome

4. Lipoatrophic diabetes

5. Others

C. Diseases of the exocrine pancreas

1. Pancreatitis

ii) 2. Trauma/pancreatectomy

3. Neoplasia

4. Cystic fibrosis

5. Hemochromatosis

6. Fibrocalculouspancreatopathy

7. Others

D. Endocrinopathies

1. Acromegaly

2. Cushing's syndrome

3. Glucagonoma

4. Pheochromocytoma

5. Hyperthyroidism

6. Somatostatinoma

7. Aldosteronoma

8. Others

E. Drug- or chemical-induced

1. Vacor

2. Pentamidine

3. Nicotinic acid

4. Glucocorticoids

5. Thyroid hormone

6. Diazoxide

7. β-adrenergic agonists

8. Thiazides

iii) 9. Dilantin

10. α -Interferon

11. Others

F. Infections

1. Congenital rubella

2. Cytomegalovirus

3. Others

G. Uncommon forms of immune-mediated diabetes

1. “Stiff-man” syndrome

2. Anti–insulin receptor antibodies

3. Others

H. Other genetic syndromes sometimes associated with diabetes

1. Down's syndrome
2. Klinefelter's syndrome
3. Turner's syndrome
4. Wolfram's syndrome
5. Friedreich's ataxia
6. Huntington's chorea
7. Laurence-Moon-Biedl syndrome
8. Myotonic dystrophy
9. Porphyria
10. Prader-Willi syndrome
11. Others

iv) IV. Gestational diabetes mellitus (GDM)

Table 2: Sign and symptoms of diabetic nephropathy

- Edema: swelling, usually around the eyes in the mornings; later, general body swelling may result, such as swelling of the legs
- Foamy appearance or excessive frothing of the urine (caused by the proteinuria)
- Unintentional weight gain (from fluid accumulation)
- Anorexia (poor appetite)
- Nauseaand vomiting
- Malaise (general ill feeling)
- Fatigue
- Headache

- Frequent hiccups
- Generalized itching

Table 3: Immunologic mechanisms in diabetic nephropathy

Stimulation of expression and synthesis of adhesion molecules, selectins, prostaglandins and fibronectin.
Development of intraglomerular hemodynamic abnormalities.
Disregulation of hyaluronan generation with the subsequent initiation of glomerular hypercellularity.
Alteration of extracellular matrix dynamics at mesangial cells and podocyte levels.
Glomerular basement membrane thickening.
Alteration of endothelial permeability.
Induction of mesangial cell proliferation.
Activation of second messenger systems, transcription factors, synthesis of cytokines, growth factors, receptors, cell adhesion molecules, enzymes involved in the synthesis of other inflammatory mediators, acute phase proteins, and major histocompatibility complex proteins.
Induction of apoptosis and necrotic cell death.
Alteration in the distribution of adhesion receptors involved in cell-cell adhesion.

Induction of reactive oxygen species.

Table 4: The potential target sites for treatment of DN (Balakumar et al., 2009).[152]

S. no.	Target sites	Therapeutic interventions
1	ACE inhibition	Captopril, lisinopril, imidapril, ramipril, perindopril, cilazapril, benazapril, trandolapril and enalapril
2	Blockade of AT1 receptor	Losartan, irbesartan, olmesartan and candisartan
3	Aldosterone antagonism	Spironolactone, eplerenone and FAD286
4	Calcium channel blockade	Nicardipine, isradipine, nitrendipine, mibefradil, verapamil, lacidipine, nifedipine and amlodipine
5	TGF-β inhibition	Anti-TGF-β2 IgG4, murine (1D11), recombinant hepatocyte growth factor (HGF), circular antisense TGF-β oligodeoxynucleotides (ODNs) and soluble human TGF-β type II receptor (sTβRII.Fc)
6	AGE inhibition	OPB-9195, ALT-946, ALT-711, aminoguanidine, TM2002 and LR-90
7	PKC inhibition	Ruboxistaurin
8	Renin inhibition	Aliskiren
9	Rho-kinase inhibition	Fasudil
10	Fibrotic inhibition	Tranilast and SMP-534
11	NADPH oxidase inhibition	Apocynin
12	PARP inhibition	INO-1001 and PJ-34
13	ETA receptor antagonism	Avosentan
14	ETA/B receptor antagonism	CPU-0213
15	Aldose reductase antagonism	Fidarestat
16	Ligands of PPAR-α	Fenofibrate and gemfibrozil
17	Ligands of PPAR-γ	Rosiglitazone and pioglitazone

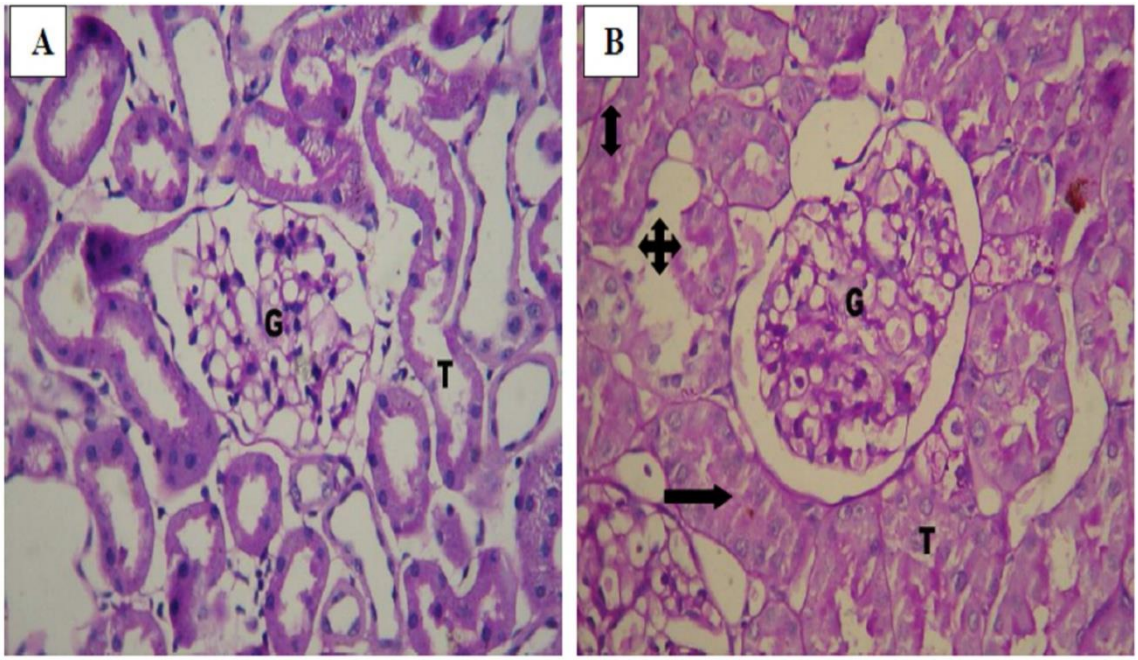

Figure 1. Representative images of PAS stained kidney, showing diabetic nephropathy in type 1 diabetes. (A) A normal histological appearance of kidney, with the normal glomerulus and tubular epithelial cells (high power-40x). (B) Renal section of diabetic control group showing glomerular hypertrophy and substantial mesangial expansion. The tubulointerstitium shows local areas of tubular atrophy, thickening of tubular basement membrane and presence of proteinaceous casts in the lumen (high power-40x). (Siddiqui et al., 2010)[173]

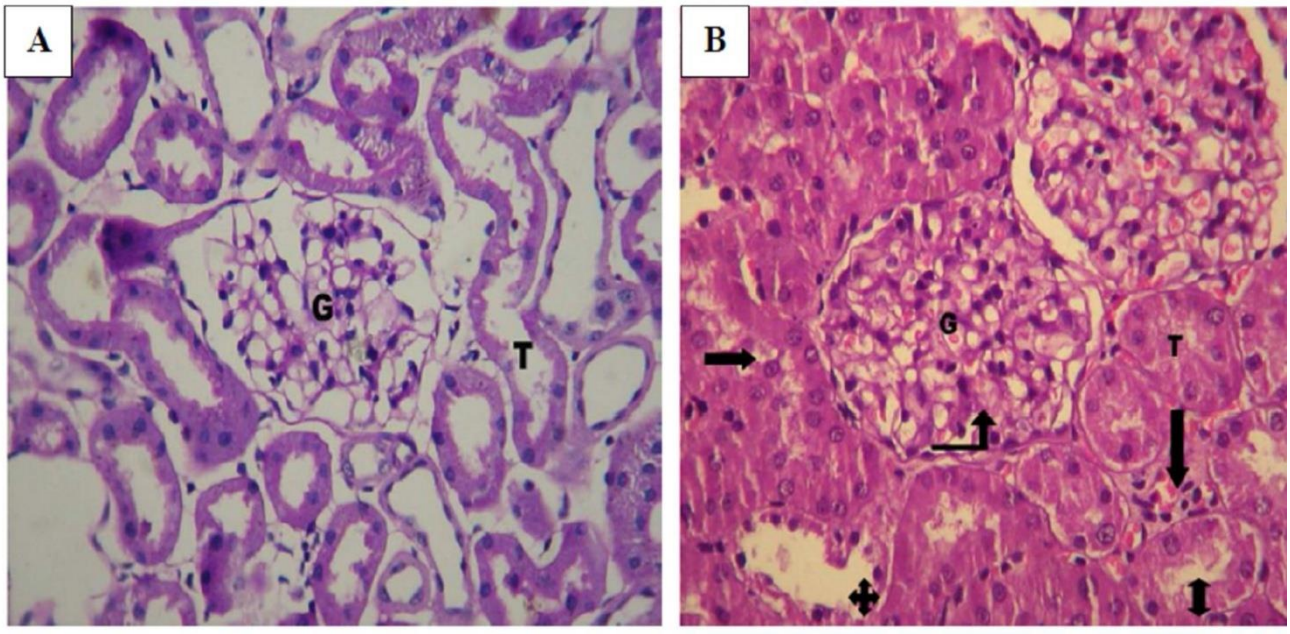

Figure 2. Representative images of PAS stained kidney, showing diabetic nephropathy in type 2 diabetes. (A) A normal histological appearance of kidney, with the normalglomerulus and tubular epithelial cells (high power-40x). (B) Renal section of HFD/STZ groupshowing substantial mesangial expansion, glomerular hypertrophy and Kimmelsteil-wilsonnodule (upward arrow). The tubulointerstitium shows infiltration of lymphocytes (down arrow),local areas of tubular atrophy (quad arrow), thickening of tubular basement membrane (doublepointed arrow) and presence of proteinaceous casts in the lumen (right arrow) (high power-40x).(Siddiqui et al; 2013)[174]