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Diabetic Nephropathy: Pathogenesis, Prevention and Treatment

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ABSTRACT

Diabetic Nephropathy (DN) is referred to as the kidney damage that occurs in people with diabetes, the earliest detectable change of which is the thickening in the glomerulus. DN is categorized into two stages; microalbuminuria and macroalbuminuria, in which the kidney leaks more serum albumin than normal in the urine. Increasing numbers of glomeruli are noted to be destroyed by progressive nodular glomerulosclerosis as DN progresses. Numerous signaling mechanisms like advanced glycation end products, enhanced reactive oxygen species generation and activation of protein kinase C have been noted to be involved in the pathogenesis of DN. Moreover, the risk factors identified in the development of DN include hypertension, hyperglycemia, hyperfiltration, smoking, advanced age and high-protein diet. Therapeutic options such as glycemic control, treatment of hypertension, hyperlipidemia are effective for preventing DN, but the numbers of diabetic patients on end-stage renal disease are still increasing and hence, therapeutic strategies preventing the pathogenesis of DN should be developed. In this paper, we review about various signaling mechanisms involved in the pathogenesis of DR. Moreover, various therapies to treat DN have been discussed in the review.

Key Words: Diabetic, Nephropathy, Signaling, Pathogenesis.

INTRODUCTION

Diabetic Nephropathy (DN) represents the most common cause of end-stage renal disease (ESRD) worldwide that accounts for the high mortality rate in patients with diabetes [1-2]. According to the World Health Organization (WHO), it is anticipated that the number of diabetes patients worldwide will grow nearby 370 million by 2030 [3-4]. In addition, it has been reported that about 20-40% of type I or type II diabetic patients will develop DN within 20-30

years of the onset of diabetes [3,5]. DN is characterized by excessive deposition of extracellular matrix (ECM) in the kidney, leading to glomerular mesangial expansion and tubulointerstitial fibrosis [6, 7]. It has been revealed that strict control of blood glucose and blood pressure significantly lowered the development and progression of DN in both type I and type II diabetes [7]. A number of risk factors have been identified in the development of DN that include genetic susceptibility, elevated blood pressure, increased blood sugar, smoking and dyslipidemia [8-10]. Moreover, various hyperglycemia-induced signaling mechanisms including increased formation of advanced glycation end products (AGEs), enhanced reactive oxygen species (ROS) generation and activation of protein kinase C (PKC), upregulation of transforming growth factor-beta 1 (TGF-β1), polyol pathway and renin-angiotensin system (RAS) contribute to the pathogenesis of DN [10-12]. In this paper, we critically review the pathophysiological role of AGEs and ROS in the pathogenesis of DN. Moreover, strategies for the prevention and treatment of DN are discussed in the article.

CLASSIFICATION OF DN

The characteristic set of structural and functional renal idiosyncrasy in patients with diabetes denotes DN [13]. The hypertrophy of the kidney, increase in glomerular basement membrane thickness, nodular and diffuse glomerulosclerosis, tubular atrophy and interstitial fibrosis represent the structural abnormalities associated with DN [14]. Moreover, the functional modifications allied to DN include an early increase in glomerular filtration rate (GFR), intraglomerular hypertension, subsequent proteinuria, systemic hypertension and eventual loss of renal function [15]. Further, it has been suggested that DN can be divided into five stages; first being the early hypertrophy stage, which is characterized by increase in renal plasma flow and GFR; second is the silent stage, which is characterized by certain morphological changes including thickening of the glomerular basement membrane, glomerular hypertrophy and tubulointerstitial expansion; third can be classified as incipient DN that can be exemplified by microalbuminuria with onset of hypertension [9-10]. The subsequent stage is overt DN which is characterized by dipstick positive proteinuria which is followed by the fifth stage which is ESRD with uremia [10,15].

RISK FACTORS OF DN

The risk factors identified in the development of DN from longitudinal and cross-sectional studies include race, genetic susceptibility, elevated blood pressure, increased blood sugar, hyperfiltration, smoking, male gender, dyslipidemia and age [8-10]. The involvement of race as the risk factor of DN has been confirmed by the fact that the incidence and severity of DN has been found to be increased in blacks, Mexican Americans, Pima Indians and Hispanics compared with Caucasians, that is attributed to their lower socioeconomic status and increased incidence of hypertension [16]. Moreover, genetic predisposition to DN is evidenced by the observation that the diabetic sibling of a DN patient showed a three-fold greater risk of developing nephropathy when compared to the diabetic sibling of a diabetic patient without nephropathy [17]. Studies have demonstrated an association between subsequent development of nephropathy and higher systemic pressures [9,18]. In addition, the presence of hypertension in the diabetic population has shown 1.5-3 fold increase than in a nondiabetic, age-matched group that further supported the contention that hypertension is a risk factor in the pathogenesis of DN [19-20]. The role of increased blood sugar level in the pathogenesis of DN is supported by the fact that DN is more likely to develop in patients with lesser degrees of glycemic control. Further, the studies showed

that the risk of development and progression of albuminuria could be substantially reduced by improving glycemic control [10,21]. Another risk factor for DN has been attributed to smoking which is associated with an accelerated loss of renal function, an increased risk for ESRD and decreased survival on commencement of dialysis [22]. An interesting study by Gall et al. showed the involvement of male gender in the development DN. The prospective observational study involving 176 patients with type-2 diabetes showed 2.6 times greater risk of developing incipient or overt nephropathy in males [23]. Furthermore, many observational studies suggested the modulatory role of lipids in the development and progression of DN. It has been reported that increase in urinary albumin excretion, higher total serum cholesterol and lower HDL cholesterol were associated with incidence of renal insufficiency, providing the evidence of the involvement of lipids in the pathogenesis of DN [24]. Astonishingly, the increasing age has been noted to be significantly associated with abnormally increased urinary albumin excretion rate in both univariate and multivariate analysis. It was found that younger age at diagnosis was significantly associated with a decrease in the estimated annual creatinine clearance in patients with type-I diabetes, that evidenced age as a risk factor in the development and progression of DN [9]. Proteinuria is considered as another risk factor in the development and progression of DN. Proteinuria>2 g/24 h has been found to be associated with a greater risk of ESRD, where an increased leakage of albumin may induce glomerular damage by the activation of inflammatory cascades [25]. Moreover, the dietary factors also play a vital role of development and progression of DN. In a study performed for 6 consecutive years in patients presented with type I and type II diabetes mellitus, it was demonstrated that those who evolved with regression of the DN presented a higher intake of polyunsaturated fatty acids and a lower intake of saturated fatty acids [10,26].

PATHOGENESIS OF DN

The resident and nonresident renal cells are stimulated by hyperglycemia and produce 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, which contribute to the pathogenesis of DN. Moreover, the development and progression of DN include various hyperglycemia-induced metabolic and hemodynamic derangements that involve increased formation of AGEs, enhanced ROS generation and PKC activation, polyol pathway and RAS (Fig 1) [10,12].

(A) AGEs and RAGE

It has been reported that the formation and accumulation of AGEs have been reported to progress at an accelerated rate under diabetes. In addition, AGEs and their signal-transducing receptor interaction have been noted to induce oxidative stress, vascular inflammation and thrombosis, thereby playing a central role in the pathogenesis of vascular complications in diabetes [27-28]. The reducing sugars react non-enzymatically with the amino groups of proteins to form reversible Schiff bases, which undergo further complex reactions such as rearrangement, dehydration and condensation to become irreversibly cross-linked, heterogeneous fluorescent derivatives termed AGEs [29]. The diabetic patients with ESRD showed approximately twice as much AGEs in tissue as in diabetic patients without renal disease, the reason for which is attributed to enhanced formation and decreased clearance of AGEs in DN patients [30]. It has also been demonstrated that AGEs stimulate monocyte chemoattractant protein-1 (MCP-1) expression in mesangial cells, which is found to be associated with monocyte infiltration in

mesangium and observed in the early phase of DN [31]. Moreover, AGEs stimulate insulin-like growth factor-I-and-II, platelet derived growth factor (PDGF) and transforming growth factor-β (TGF-β) in mesangial cells, that has been noted to play an important role in the pathogenesis of glomerulosclerosis and tubulointerstitial fibrosis in DN [32-33].

Receptors for AGEs (RAGE) are signal transducing receptor for AGEs that mediate the inflammatory reactions evoked by AGEs [28,34]. In humans, the expression of RAGE is enhanced in podocytes and mesangial cells in diabetic patients with nephropathy [35]. Furthermore, accumulating evidence suggest the modulatory role of AGE-RAGE axis in the pathogenesis of DN that is evidenced by several animal studies which also supported the crucial role for RAGE in the development and progression of DN [36]. The activation of RAGE in podocytes also contributed to the expression of vascular endothelium growth factor (VEGF) and enhanced activation of inflammatory cells in the diabetic glomeruli, causing albuminuria and glomerulosclerosis in diabetes. In addition, experimental studies have shown that hyperglycemia-induced RAGE activation developed renal changes seen in human diabetic nephropathy such as glomerular hypertrophy, glomerular basement membrane thickening, mesangial matrix expansion, connective tissue growth factor (CTGF) overexpression and nuclear factor kappa B (NF-κB) activation [37-38].

(B) Mitochondrial ROS

A key role in the pathogenesis of DN has been attributed to hyperglycemia-induced enhanced mitochondrial ROS production which is considered as an important mediator of vascular complications in diabetes [39]. The hyperglycemia has been noted to generate ROS as a result of glucose auto-oxidation, metabolism and formation of AGEs [40-41]. In addition, it has been demonstrated that ROS-mediated renal cell apoptosis is induced by hyperglycemia, angiotensin II, TGF-β and albumin [42-43]. It has been noted that mitochondria-derived ROS constitute the major source of intracellular ROS that results in oxidative damage of proteins, lipids and DNA, ultimately leading to apoptosis and renal injury [44-45]. Many renal cell types like mesangial cells, endothelial cells and tubular epithelial cells have been found to produce high levels of ROS under hyperglycemic conditions [46]. ROS has been noted to activate several pro-inflammatory transcriptional factors, resulting in the production of cytokines, chemokines and vascular adhesion molecules that subsequently lead to the influx of inflammatory cells into the kidney. This formation of ROS-mediated renal inflammation further aggravates ROS-mediated cell injury, apoptosis and kidney dysfunction. The contributors to ROS formation in the diabetic kidney are noted to be the superoxide producing enzyme NADPH oxidase (NOX), and xanthine oxidase, which catalyzes oxidation of hypoxanthine [39]. In addition, hyperglycemia-mediated sudden production of ROS and reduced anti-oxidant capacity induces apoptosis that leads to renal injury and DN which is supported by the fact that in diabetes patients a combination of increased ROS formation and diminished anti-oxidant defense results in apoptosis of renal cells. The hyperglycemia-mediated mitochondrial ROS formation has been further noted to activate proapoptotic p38 MAPK and caspase-3 activation [47]. Different caspases like caspase-3, 9 and 12 play a crucial role in hyperglycemia-induced apoptosis of proximal tubular epithelial cells ultimately leading to the progression of DN [48]. In renal tubular epithelial cells hyperglycemia-induced oxidative stress leads to an increased Bax protein expression accompanied by a reduced Bcl-2 expression leading to the apoptosis of renal tubular cells [49]. Furthermore, hyperglycemia-induced ROS generation has been noted to stimulate RAS gene expression in

renal proximal tubular cells that further confirmed the modulatory role of ROS in the development and progression of DN [50-51]. Moreover, the AGE-RAGE-mediated ROS generation stimulates production of pro-sclerotic growth factors such as TGF-β and CTGF via mitogen-activated protein kinase (MAPK) and PKC pathways in both mesangial and renal tubulointerstitial cells [52-53]. ROS-induced production of TGF-β1, the key regulator of ECM remodeling, causes mesangial expansion and tubular epithelial-mesenchymal transition leading to tubulointerstitial fibrosis, providing the proof of role of ROS in the progression of DN [54]. Additionally, ROS has been noted to activate various transcription factors like NF-κB and activated protein-1 (AP-1) leading to upregulation of genes and proteins involved in the development and progression of DN.

(C) Additional factors

Other factors that appear to have a role in the pathogenesis of DN are obesity and release of proinflammatory factors and growth factors, which have been documented to produce glomerular hyperfiltration due to reduction in the resistance of the afferent and efferent glomerular arterioles and consequent increased renal perfusion [55-56]. Moreover, the levels of endothelin 1 (ET-1), an important vasoconstrictor, in plasma were progressively higher in the DN patients providing the certainty of involvement of ET-1 in the pathogenesis of DN [57]. Albuminuria has been noted to activate a series of inflammatory pathways through tubular cells in order to exacerbate DN [58]. In addition, the mechanical stress resulting from renal hyperperfusion induces the release of cytokines (TNF-α), growth factors (VEGF, TGF-β1), cholesterol and local triglycerides that further induce the accumulation of proteins from extracellular matrix, leading to mesangial expansion and glomerulosclerosis [10]. Furthermore, a sequence of circulating markers of inflammation such as C reactive protein (CRP), TNF and interleukin (IL)-1,6 and 18 are increased in DN whose levels show a relationship with albuminuria and progression of ESRD. Hyperglycemia-mediated oxidative stress induced actvation of angiotensin II, type IV collagen and fibronectin has contributed to progressive glomeruloesclerosis [59]. Additionally, the inflammatory factors such as accumulation of macrophages in the tubular interstice have also been noted to be involved in the development of tubulointerstitial lesion. Macrophages produce free radicals, inflammatory cytokines and proteases that induce tubular damage in DN patients providing the evidence of modulatory role in development and progression of DN [10,58].

PREVENTION AND TREATMENT OF DN

The principles of prevention and treatment of DN are similar, depending on the stage and severity of the disease and thus, it becomes imperative to define the DN stage which is the target of intervention. However, the objective to be pursued is retarding the development or progression of DN and to decrease the patients' mortality and morbidity. ACE inhibitors and angiotensin receptor blockers (ARBs) appeared to be successful in reducing the proteinuria and decreasing the creatinine doubling rate [60]. Recently, studies have been designed in order to evaluate the benefit of intensive glycemic control in large sets of patients which showed protective effect on the development and progression of albuminuria [61]. Another choice of therapy for the prevention and treatment of DN is RAS blockade with ACE inhibitors or ARB which confers an additional benefit on renal function independent of BP reduction [15]. These drugs decrease urinary albumin excretion (UAE) and the rate of progression from microalbuminuria to more advanced stages of DN and hence, the combination of these classes of drugs has been proposed as an alternative to treat DN. Another step that has been proposed is the

blockage of aldosterone action, where the addition of spironolactone, an aldosterone antagonist, to ARBs or ACE inhibitor showed promising results in reducing UAE and proteinuria in chronic kidney disease in type II diabetic patients than each drug alone [62]. Moreover, the dual blockage of the renin-angiotensin-aldosterone system with a direct renin inhibitor, aliskiren, and losartan at maximal recommended dose of 100 mg daily showed a greater reduction in proteinuria in patients presented with DN [63]. Further, sulfonylureas and glitazones have also been reported to be used in chronic renal disease with stages 3, 4 and 5 that confirm their potential as a therapeutic adjuvant in the treatment and prevention of DN [64-65]. In addition, dietary intervention represents as an alternate in the prevention and treatment of DN, which is evidenced by a meta-analysis of studies performed with type I and II diabetes mellitus patients and clinical nephropathy, in which the dietary protein restriction retarded DN progression confirming the significance of dietary intervention in the prevention and treatment of DN [10,66].

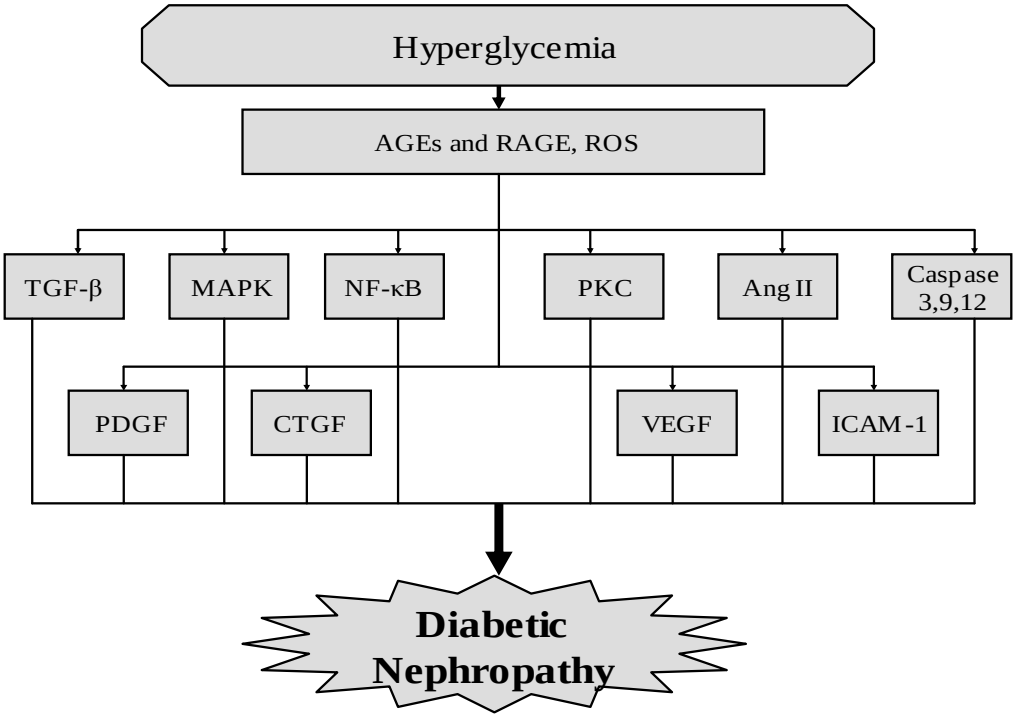

Figure 1. Diagram showing hyperglycemia-induced biochemical alterations in the pathogeneis of DN
AGE: Advanced glycation end products; RAGE: Receptors for AGEs; ROS: Reactive oxygen species; TGF-β-Transforming growth factor-beta; MAPK-Mitogen-activated protein kinase; NF-κB: Nuclear factor kappa B; PKC: Protein kinase C; Ang II: Angiotenisn-II; PDGF: Platelet derived growth factor; CTGF: Connective tissue growth factor; VEGF: Vascular endothelium growth factor; ICAM-1: Imtracellular cell adhesion molecule.

CONCLUSION

DN has been considered as the leading cause of ESRD in developed countries whose prevalence is continuously increasing in the developing countries. Further, DN is a chronic complication of DM with a growing incidence and hence, it is essential to have a better understanding of it so as

to provide prevention and management to avoid progression of DN. However, current therapeutic options are far from satisfactory, and the numbers of diabetic patients with ESRD are still increasing in industrialized countries. Thus, the development of novel therapeutic strategies that could specially target DN are immediately considered necessary.

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