INTRODUCTION

Rapid urbanization and industrialization has produced social and economic advancement in developing countries like India which has resulted in dramatic lifestyle changes and lifestyle related diseases. The transition from a traditional to modern lifestyle, consumption of diets rich in fat and calories combined with high level of mental stress has compounded the problem further. This has laid to increased prevalence of metabolic disorders such as diabetes mellitus (DM) in India. There are several studies from various parts of India which reveal a rising trend in the prevalence of DM in the urban areas. [1]

There is a worldwide epidemic of DM, obesity, and metabolic syndrome, created by combination of genetic and environmental factors. [2] With largest number of DM patients in the country, India has earned a distinction of “Diabetes capital of the world”. [3] DM has afflicted humankind from time immemorial, with its earliest medical documentation recorded over 3,000 years ago. However, it is only in the past century that the treatment and course of DM has undergone a radical change. With introduction of insulin therapy in 1921, DM was transformed from an imminently lethal disease to a chronic disease. [4]

The term DM describes a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or action. [5]

In 2003, the dramatic increase in DM population was observed worldwide, and it is likely to increase more rapidly in future. About 5.1% of the world population i.e. around 194 million people were likely to have DM globally. The projected number of DM patients by year 2025 is around 330 million worldwide, and will be endemic in Asia. Over the next 25 years, India alone is expected to see an increase from 36 to 73 million people with DM and majority of the cases being type 2 DM. [6, 7]
The vast majority of DM patients are classified into one of two broad categories: type 1 diabetes mellitus (T1DM), which is caused by an absolute deficiency of insulin, and type 2 diabetes mellitus (T2DM), which is characterized by insulin resistance with an inadequate insulin secretion. In addition, women who develop DM during their pregnancy are classified as having gestational DM. Finally, there are a variety of uncommon and diverse types of DM, which are caused by infections, drugs, endocrinopathies, pancreatic destruction, and genetic defects. These unrelated forms of DM are included in the “Other Specific Types” and classified separately. [8]

As the hyperglycemia develops gradually and it is not severe enough to produce the classical symptoms in early stages of the disease, T2DM patients remain undiagnosed for many years, due to which these patients are at higher risk of developing micro and macro vascular complications with high morbidity and mortality. The manifestations include the metabolic syndrome, a cluster of cardiovascular (CVD) risk factors, which apart from glucose intolerance include dyslipidemia, hypertension, visceral obesity, hypercoagulability and microalbuminuria. [9]

A higher proportion of individuals with T2DM are found to have microalbuminuria and overt nephropathy shortly after the diagnosis of their DM, because DM is present undiagnosed for many years before the diagnosis is made. [9] Diabetic nephropathy (DN), also known as Kimmelstiel-Wilson syndrome or nodular DM glomerulosclerosis or intercapillary glomerulonephritis, is a clinical syndrome characterized by albuminuria (>300 mg/day) confirmed on at least two occasions 3-6 months apart, permanent and irreversible decrease in glomerular filtration rate (GFR), and arterial hypertension. [10]

The earliest clinical evidence of nephropathy is the appearance of low but abnormal levels (≥30 mg/day) of albumin in the urine, referred to as microalbuminuria. Once overt nephropathy occurs, without specific
interventions, the GFR gradually falls over a period of several years at a rate that is highly variable from individual to individual. [9]

It is an irony of medical progress that the renal involvement in T2DM had been considered in past as a benign condition for the kidney without causing renal function loss greater than expected from the “normal” aging process. [11] Before the advent of insulin therapy, diabetic nephropathy was essentially an unknown entity, but as World War II loomed on the horizon, so did the beginnings of the equally devastating epidemic of diabetic ESRD. [12] Irrespective of the etiology of hyperglycemia, 20-30% of DM patients develop nephropathy and are associated with a high incidence of ESRD. [9, 12] DM has become the most common single cause of ESRD; this is due to the facts that 1) DM, particularly T2DM, is increasing in prevalence; 2) DM patients now live longer; and 3) patients with diabetic ESRD are now being accepted for treatment in ESRD programs where formerly they had been excluded. [9, 11, 13] Pathogenesis of DN is very complicated and results from the interaction of hemodynamic and metabolic factors. The evidence from in vitro studies shows that hyperglycemia has a direct effect on mesangial cell proliferation, matrix expansion, and glycosylation of glomerular proteins. [14, 15]

Increasing evidence also showed the importance of increased oxidative stress due to high glucose induced reactive oxygen species (ROS) generation in the pathogenesis of DN. Although the ROS production may be influenced by numerous mechanisms, the most important role in their production is played by superoxide produced by glycolysis and oxidative phosphorylation in the mitochondria. ROS activate all important pathogenic mechanisms, such as increased production of proteins modified by glucose or glucose-derived products such as methylglyoxal, i.e. amadori products, and advanced glycation end products (AGEs), by increased glucose entry into the polyol pathway, and PKC activation, and subsequently mitogen activated protein kinases (MAPK).
In addition, ROS directly damage endothelial glycocalyx, which leads to albuminuria without the concurrent damage to the glomerular basement membrane (GBM) itself. [16]

Renal hypertrophy is an early event; irreversible changes such as glomerulosclerosis and tubulointerstitial fibrosis are preceded by hypertrophy. Parallel to and to some extent concomitant with renal hypertrophy, hyperfiltration and intrarenal hypertension develop in type 1 as well as in T2DM. Both hemodynamic and structural changes are important and are interrelated. Also, shear stress and mechanical strain, resulting from altered glomerular hemodynamics and glomerular hypertension, induce the autocrine and/or paracrine release of cytokines and growth factors, which in turn plays a role in genesis of glomerulosclerosis and interstitial fibrosis. [11]

Apart from the individual human suffering that cannot be expressed in numbers, patients with T2DM undergoing dialysis consume significantly more financial resources than those with non-diabetic ESRD. [11] In the U.S., DN accounts for about 40% of new cases of ESRD, and is expected to incur yearly costs of 18 to 30 billion dollars over the next decade. [9, 12]

It is estimated that in India about 1,00,000 people suffer from ESRD annually, of which only about 20,000 get treated. [17] DM patients with chronic kidney disease (CKD) prior to ESRD spend more hospitalizations than patients without any complications. Almost 70% of Indian population is in below-poverty-line category, and although the cost spent on dialysis is comparatively cheaper than other countries, 90% of the Indians cannot afford it. [18]

Dyslipidemia plays an important role in the progression of kidney disease in patients with DM. The changes in lipoproteins, specifically triglyceride rich lipoproteins, cause damage to kidney. Lipids cause renal injury by oxidative stress. Lipoprotein abnormalities are more common in DN patients and contribute to coronary heart diseases in DN patients. The characteristic diabetic dyslipidemia associated with insulin resistance includes hypertriglyceridemia
and high levels of very low density lipoprotein cholesterol (VLDL-C) and low levels of high density lipoprotein cholesterol (HDL-C) [19,20]. A high concentration of triglyceride-rich VLDL particles inhibits insulin binding to its receptor and affects insulin action. [21]

Insulin has effect on the activity of lipoprotein lipase (LPL) [22]. Thus, diminished LPL activity may reduce VLDL catabolism and increased hepatic VLDL triglyceride secretion and hepatic apoB-100 production. Secretion and increased flux of free fatty acids to liver further lead to accumulation of triglycerides. [23]

Insulin has profound effects on HDL metabolism. Low levels of HDL-C and apoA-I with high ratio of total cholesterol to HDL-C are strongly related to insulin resistance and are unique to patients with T2DM.

The increase in low density lipoprotein cholesterol (LDL-C) in case of DM with or without nephropathy with poor glycemic control is due to glycation of LDL and oxidative modification of LDL-C. Oxidized modified lipoproteins are the direct mediators of glomerular injury which progresses to DN. [24, 25]

Lipoproteins are taken up by mesangial and epithelial cells of glomeruli. Mesangial cells express scavenger receptors which uptake the glycosylated and oxidized LDL, then the mesangium itself is taken up by infiltrated glomerular monocytes which are activated and converted into macrophages. This stimulates secretion of various chemotactic factors and adhesion molecules. These factors result in monocyte infiltration which plays an important role in the pathogenesis of glomerulosclerosis and tubular fibrosis in DN. The uptake of modified LDL by mesangial macrophages also stimulates the eicosanoid synthesis such as thromboxanes and leukotrienes leading to potentially harmful effect in intraglomerular hemodynamics [26-29].

Lipid abnormalities associated with DM and DN include high plasma levels of TG, VLDL-C, IDL-C, LDL-C and low concentrations of HDL-C. In case of
poor glycemic control in DN, total cholesterol is increased due to accumulation of LDL-C. [13]

**Angiotensin converting enzyme (ACE) Insertion/Deletion (I/D) polymorphism:**

A positive family history is clinically useful to identify patients with T2DM at high renal risk. [30] Strong evidence suggests that genetic predisposition plays a significant role in development of DN which clusters within families in both type 1 and T2DM. [31, 32]

The hemodynamic changes may be explained in part by alterations in the renin-angiotensin system (RAS). [32] The RAS may play important role in the development of DN. Genetic studies have revealed that the genes of RAS are highly polymorphic, and have been suggested as potential risk factors in addition to environmental factors. These alter the genetic make-up of RAS and affect the status of RAS in individuals. ACE gene consists of 26 exons and spans 21kb on chromosome 17. The I/D polymorphism in ACE gene is characterized by presence or absence of 287-bp fragment, within intron 16 and it has been studied extensively with renal [9] and CVD [10] complications of DN. It may play a potential role in the development of DN. Deletion polymorphism is associated with elevated activity of ACE. [31, 32]

Most studies confirmed that ACE I/D polymorphism is involved in the susceptibility to overt nephropathy with protective role of ACE II genotype against the disease in both T1DM and T2DM. [33] In diabetic kidney disease, increase in tissue angiotensin II leads to an increase in oxidative stress, intraglomerular pressure, glomerular hyperfiltration, endothelial damage, thrombosis, inflammation and vascular remodeling. Thus, pathology of DN starts with various renal functional changes including glomerular hyperfiltration and hyperperfusion coupled with increased GFR. [31, 33]

The reports indicating increased activity of serum angiotensin-I converting
enzyme in individuals with DD and ID genotypes help in determination of ACE genotypes of DN patients which may be beneficial in assessing prognosis of DN to ACE inhibition therapy. Patients with II genotype are likely to have a favorable progress and good response to ACE inhibitors, whereas DN patients with DD and ID genotypes may progress to ESRD at a relatively faster rate and the response to ACE inhibitors may not be as favorable as in those with II genotype. [34]

**Gene expression of podocyte associated proteins:**

In mammalian tissues, mRNA expression, related to the transcriptional activity of various genes, is in a state of dynamic turnover during different developmental and pathophysiological states. In such a process, various genes are differentially up- or down-regulated depending on a given disease process. The isolation of such genes not only would lead to the identification of candidate disease-susceptible or resistant genes, but also would delineate novel mechanisms and pathways that are involved in various diseases. [35]

The integration of gene expression data into their functional context is one of the greatest challenges to employ this information towards “personalized molecular medicine” of DN. Genes influenced by a common environmental challenge or genetic predisposition are assumed to show co-regulation in the examined tissue resulting in similarities of their patterns of expression in these patients. [36]

Glomerular visceral epithelial cells, namely podocytes, are highly specialized cells and give rise to primary processes, secondary processes, and finally foot processes. The foot processes of neighboring podocytes inter-digitate, leaving between them filtration slits. These are bridged by an extracellular substance, known as the slit diaphragm, which plays a major role in establishing size-selective barrier to protein loss. Furthermore, podocytes are known to synthesize matrix molecules to the glomerular basement membrane (GBM), including type IV collagen, laminin, entactin, and agrin. The hemodynamic
changes, and local growth factors such as transforming growth factor-beta and angiotensin II, which are considered mediators in the pathogenesis of DN, induce hypertrophy, apoptosis, and structural changes directly or indirectly, and increase type IV collagen synthesis in podocytes. [37]

As podocyte proteins are specifically expressed on podocyte cells, analyzing their expression has increased our understanding of pathogenesis of proteinuria in DN. Slit diaphragm podocin is closely linked to cytoskeletal alpha actinin-4 and synaptopodin, enabling dynamic rearrangements of the podocyte architecture. Podocalyxin at the apical membrane, a phenotypic marker of the podocyte cell, limits the passage of negatively charged albumin. [38]

The expression of podocyte markers in glomerular disease has been investigated using immunostaining or molecular analysis to quantitatively measure protein and mRNA levels, respectively, in renal tissue. However, renal biopsy is usually not indicated for the diagnosis of DN. Recently, the detection of increased urinary mRNA of podocyte-specific molecules in DN and other glomerular diseases has emerged as a useful, non-invasive tool to assess podocyturia as a signal of podocyte damage and disease activity. [39]

Peripheral blood mononuclear cells (PBMCs) generally refer to monocytes and lymphocytes, representing cells of the innate and adaptive immune systems. PBMCs are a promising target tissue in the field of nutrigenomics (to identify the genes that influence diet related diseases such as T2DM and CVD) because they seem to reflect the effects of dietary modifications at the level of gene expression. The use of transcriptomics can help to provide more information about eventual biomarkers of certain diseases or physiological changes related to the pathogenesis of the disease. PBMCs are convenient because they can be easily collected in sufficient quantities. PBMCs have been used for exploring gene expression in various diseases and to predict clinical outcomes. [40]

This indicates that there is a need for further studies on biochemical and molecular aspects of T2DM patients with nephropathy. With this background,
we hypothesized that ACE I/D gene polymorphism may play an important role in the pathogenesis of DN and it may be associated with CVD markers. Differences in the expression levels of podocyte proteins viz. podocalyxin, podocin and synaptopodin, from diabetic subjects with nephropathy and its comparison with T2DM patients without nephropathy and healthy individuals from peripheral blood mononuclear cells may be used as early kidney damage markers.