REVIEW OF LITERATURE

India is the second most populous country in the world, accounting for 15% of the world population. [41] This large population of India poses a major challenge, as the number of DM patients would be high even with a low prevalence rate. This is particularly true because a large number of individuals could have undetected DM. [42]

DIABETES MELLITUS

DM is the most common endocrine disorder, about 300 million people worldwide will subsequently have the disease by 2025. [43, 44] DM is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism, resulting from pancreatic damage; leading to defective insulin production and/or because of insulin resistance. [45]

The effects of DM include organ damage, dysfunction, and failure over a period of time, starting from the eyes, kidneys, heart, neural tissue, and blood vessels. If DM is undiagnosed or poorly controlled, it can lead to severe complications such as obesity, neuropathy, nephropathy, retinopathy, cardiopathy, osteoporosis and coma leading to death. [46, 47]

HISTORY

The best early evidence of a description of the symptoms of DM in the world’s literature is recorded in the Ebers papyrus that appears to date from 1550 BC. [48] Two Greek Physicians in the Roman era, Galen (AD 130-201), who practiced in Rome, and Arateus of Cappadocia, delineated the disease further. Arateus coined the term ‘diabetes’, meaning “Siphon”, to explain the “liquefaction of the flesh and bones into urine”. [49]

In 1675, Thomas Willis, added the word mellitus, from the Latin meaning “honey” with reference to the sweet taste of the urine. [50] Sushruta (6th century BC) identified DM and classified it as “Madhumeha”. DM is first recorded in English, in the form DM, in a medical text written around 1425. [51, 52]
The discovery of the role of pancreas in DM is generally ascribed to Joseph von Mering & Oskar Minkowski, in 1889. In 1910, Sir Edward Albert Sharpey-Schater suggested deficiency of a single chemical, produced by the pancreas, ‘insulin’ (Latin insula→island). [50, 53]The distinction between what is now known as T1DM&T2DM was first clearly made by Sir Harold Percival (Harry) Himsworth, and published in January 1936. [54] Some of the milestones in research and drug development for diabetes are mentioned below:

- Development of Metformin in 1922 for treatment of T2DM
- Development of long acting insulin NPH in 1940s by Novo-Nordisk
- Identification of first of the sulfonylureas in 1942
- Reintroduction of the use of biguanides for T2DM in late 1950s. The initial phenformin was withdrawn worldwide (in the U.S. in 1977) due to fatal lactic acidosis and metformin was first marketed in France in 1979, but not until 1994 in the US.
- The determination of the amino acid sequence of insulin (by Sir Frederick Sanger, for which he received a Nobel Prize). Insulin was the first protein for which amino acid sequence was determined.
- The radioimmunoassay for insulin, as discovered by Rosalyn Yalow and Solomon Berson (gaining Yalow the 1977 Nobel Prize in Physiology or Medicine).
- The three-dimensional structure of insulin (PDB: 2INS).
- Dr. Gerald Reaven's identification of the constellation of symptoms now called metabolic syndrome in 1988.
- Demonstration that intensive glycemic control in T1DM reduces chronic side effects as glucose levels approach 'normal' in a large longitudinal study, and also in T2DM in other large studies.
- Identification of the first thiazolidinedione as an effective insulin sensitizer during the 1990s. [54]
CLASSIFICATION OF DIABETES MELLITUS

Several forms of DM have been defined. Scientists are still searching for new forms of DM with some variations and establishing their prevalence in the population. The etiological classification of DM was recommended by World Health Organization (WHO) and American Diabetes Association (ADA). DM is divided into four main types. [55, 56]

**Type 1 Diabetes mellitus**

T1DM is an autoimmune disease leading to destruction of pancreatic β-cells and absolute insulin deficiency as a result; it leads to a type of DM in which insulin is required for survival. It is a common disease found in children and adolescents. Individuals with T1DM are metabolically normal before the disease is clinically manifested, but the process of β-cell destruction can be detected earlier by the presence of certain autoantibodies. The β-cells of langerhans of pancreas are mistakenly destroyed by autoantibodies by the immune system. [55, 56]

**Type 2 Diabetes mellitus**

T2DM is commonest of all forms of DM, in developed countries it accounts for about 85 to 95% of cases and higher number of T2DM is seen in developing nations. The process of disease development and progression take years or decades. It is usually antecede by pre-diabetes, in this condition the blood glucose level is in between upper normal but not higher enough to label the person as diabetic. The onset of disease is usually after 40 years of age, but now a days it is also seen in children. [55, 56]

**Gestational Diabetes mellitus**

Gestational DM occurs in pregnant women who have never suffered from DM, but experience gestational hyperglycemia, which might be transient or may develop into T2DM after pregnancy. It is a temporary condition due to metabolic alterations that any previously non diabetic pregnant women can develop mostly during the third trimester. The changes in hormone levels during
pregnancy is the important factor for this disease, along with increased weight and family history of DM. [55, 56]

Other types of Diabetes mellitus

Other specific types of DM are those in which the underlying defect or disease process can be identified in a relatively specific way or those that have other distinctive, distinguishing features. This category encompasses a variety of types of DM secondary to other specific conditions or associated with particular diseases or syndromes with a distinct etiology. [55, 56]

Secondary diabetes

Secondary DM may develop due to a range of diseases such as pancreatitis, cystic fibrosis, Down’s syndrome and hemochromatosis as well as the use of drugs including corticosteroids, other immunosuppressive drugs, diuretics and pancreatectomy. [55, 56]

TYPE 2 DIABETES MELLITUS

T2DM is thought of as a disease of the middle aged and elderly, with typical onset after 40 years. [57] However, it is frequently seen in children and adolescents attributed to rising obesity rates due to alterations in dietary patterns as well as life style during childhood. [58]

T2DM is the most common form of DM, and is a disorder that is characterized by hyperglycemia in the context of insulin resistance and relative insulin deficiency, either of which may be the predominant feature. Usually both are present at the time DM becomes clinically manifested. The classic symptoms are excess thirst, frequent urination, and constant hunger. [46, 59]

T2DM frequently goes undiagnosed for many years because the hyperglycemia develops gradually and in the earlier stages is not severe enough to produce the classic symptoms of DM; however, such patients are at increased risk of developing macrovascular and microvascular complications. [56, 60] Long-term complications from high blood sugar can include heart disease, stroke, retinopathy where eyesight is affected, kidney failure which may
require dialysis, and poor circulation of limbs leading to amputations. [61]

**Epidemiology of Diabetes mellitus**

The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 285 million in 2010. [62] It is estimated that by the year 2030, the global prevalence of DM for all age-groups will be 4.4% or 366 million people. [63] The region most likely to experience the main brunt of the epidemic is Asia. [57]

India has the largest number of DM population in the world and it is estimated that almost 41 million Indians are diabetic, and that figure is expected to reach 73 million by 2025. [63] The incidence is higher in the south than in the north, particularly in cities such as Chennai and Hyderabad, where about 16% of the population is diabetic. [64] The various studies conducted in different states mentioning the respective prevalence is shown in figure 1.

![Figure 1: Major Studies Conducted in India [65]](image-url)
The top three countries in terms of the number of T2DM individuals with DM are India (31.7 million in 2000; 79.4 million in 2030), China (20.8 million in 2000; 42.3 million in 2030); and the US (17.7 million in 2000; 30.3 million in 2030). [66]

**Signs and symptoms**

The classical symptoms of DM are polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Symptoms may develop quite rapidly (weeks or months) in T1DM while in T2DM they usually develop much more slowly and may be subtle or completely absent. The Overview of the most significant symptoms of DM is depicted in Figure 2. [67]

![Figure 2: Overview of the most significant symptoms of DM](image)

**Pathophysiology of Type 2 Diabetes mellitus**

T2DM is characterized by the combination of disturbances in insulin secretion by pancreatic β-cells and peripheral insulin resistance, which is often related to obesity. Insulin resistance is caused by defects in the signaling pathways that
process the insulin signal in its target tissues. [69]

There is a strong link between insulin secretion and insulin sensitivity, and any change in one of these two produces adaptation in the other. There is no consensus on which one of the two abnormalities is the primary defect in the development of T2DM. It is well documented that T2DM develops when the pancreas is unable to secrete more insulin to compensate for existing insulin resistance. This is in accordance with an observation that insulin resistance is present early in the natural history of T2DM, whereas marked beta cell dysfunction is a rather late event. Both impaired insulin secretion and insulin resistance are influenced by genetic and environmental factors. [70]

**COMPLICATIONS OF TYPE 2 DIABETES MELLITUS**

The impact of DM on both the individuals’ health and on the health care systems, resides almost entirely in the long term “complications” of DM affecting almost every system in the body including eyes, kidneys, heart, feet and nerves. [71] The Overview of the complications of DM is depicted in Figure 3. DM related complications can be broadly classified as:

1. **Microvascular complications:** Affecting the retina (diabetic retinopathy), kidney (DN), and the peripheral nerves (diabetic neuropathy).

2. **Macrovascular complications:** Affecting the heart (CVD), brain (cerebrovascular disease), and the peripheral arteries (peripheral vascular disease). [71]
MICROVASCULAR COMPLICATIONS

People with DM have an increased risk of developing microvascular complications, which if undetected or untreated, can have a devastating impact on quality of life and has a significant burden on health care system. The most specific complications of DM are microvascular complications. [73]

Diabetic retinopathy (Vision)

DM results in characteristic lesions in the retinal blood vessels. Diabetic retinopathy is still the most common cause of acquired blindness in the Western world. Its prevalence increases most steeply between 5 to 15 years of DM duration, being about 60% after 20 years in the European population. An excess of glucose activates the polyol pathway, which causes accumulation of sorbitol in the lens and is accompanied by cataracts. The etiology of retinopathy includes hyperglycemia-associated biochemical, anatomical and functional changes. [74]
Diabetic nephropathy (Chronic Kidney Disease)

DN is estimated to develop in one third of the patients in both main types of DM. Nephropathy is characterized by GBM thickening and arteriosclerosis of small arterioles. The mechanisms proposed to induce glomerulosclerosis include hyperglycemia, hyperfiltration related increase of glomerular pressure, and increased blood viscosity. [9, 75, 76] The hallmark of renal damage in DM is increased excretion of albumin in the urine. Nephropathy may culminate in uremia and, in fact, most of the hemodialysis patients and the patients receiving renal transplants have DM. [77]

Diabetic neuropathy

The term diabetic neuropathy includes either a clinical or subclinical disorder without any additional causes of peripheral neuropathy other than DM. [78] It may affect both sensory and autonomic nerves, but distal symmetric polyneuropathy is probably the most common consequence which, together with peripheral vascular disease, is an important etiologic factor for foot ulcerations and lower limb amputations. [74]

MACROVASCULAR COMPLICATIONS

Macrovascular complications are considered to be a deadly triangle, comprising CVD, peripheral vascular disease and cerebrovascular disease. [79] The presence of DM, in addition to any or all the other risk factors (such as smoking, hypertension, dyslipidemia, and genetic factors), approximately doubles the probability of developing macrovascular diseases. [74]

Cardiovascular disease

Macrovascular complications of DM are due to accelerated atherosclerosis and have an important role in the increased morbidity and mortality of these individuals. [80] People with DM are at a high risk of suffering from CVD, peripheral vascular disease and CVD complications such as myocardial infarction, coronary heart failure, and stroke and these chronic illnesses take 10–20 years to manifest. [81]
Peripheral vascular disease

Peripheral vascular disease in DM patients differs from that in non-DM individuals. In non-DM individuals the sites of occlusion are more proximal, usually the infra-renal aorta, iliac and superficial femoral arteries, with sparing of distal vessels whereas in DM patients, occlusive lesions occur in more distal vessels such as the tibials and peroneals. [82, 83]

Cerebrovascular disease

Stroke is the third commonest cause of mortality after heart disease and cancer and represents a major health burden in India. [84] Patients with DM have a higher frequency of stroke and also poor prognosis after a stroke. The risk of stroke is more than double among T2DM individuals as compared to general population. [85] Though the prevalence of stroke varies greatly in various reports, there has been a definite increase in its prevalence and incidence in India over last 30 years. [86]
DIABETIC NEPHROPATHY:

DM is the most frequent cause of chronic kidney failure in both developed and developing countries, and existing trend suggest that the number will go on increasing in the future, this may be due to 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, 87, 88]

DN, also known as Kimmelstiel Wilson syndrome or nodular diabetic glomerulosclerosis or intercapillary glomerulonephritis, is a clinical syndrome characterized by albuminuria (>300 mg/day or >200 mcg/min). [89] DN is a late chronic microvascular complication of both T1DM and T2DM, occurring over the years in vulnerable people after 15 to 25 years of DM. [87, 90]

![Figure 4: DN causes the damage of glomerulus, kidney disease. [91]](image)

About 20-30% of both DM patients are more likely to develop DN, and family related studies confirmed the genetic factor has an important role in development of DN. DN a clinical syndrome is manifested by presence of albuminuria, consistently decreasing GFR, and increased risk for CVDs. [9, 88]

In the United States, about 40% of new cases of ESRD are found to be caused by DN, and costed excess of $15.6 billion for the treatment of DM patients with ESRD. [9]
Definition of Diabetic Nephropathy:
DN or diabetic kidney disease is defined by characteristic structural and functional changes, with the predominant structural changes being mesangial expansion, GBM thickening, and glomerular sclerosis. Functional characteristics include hyperfiltration, microalbuminuria, and macroalbuminuria with incipient progressive proteinuria which is often followed by a slowly progressive decline in GFR and, over time, ending in symptomatic ESRD requiring renal replacement therapy. [92]

Definitions related to diagnosis of Diabetic Nephropathy [9]

Proteinuria: Urinary protein >0.5g/24 hours Albumin/creatinine ratio (ACR) >30mg/mmol.

Albuminuria: Urinary albumin excretion rate >300 mg/day or >200 µg/min.

Microalbuminuria: Urinary albumin excretion rate 30–300 mg/day or 20–200 µg/min. ACR ≥3.0 mg/mmol

DN is the leading cause of ESRD worldwide. [93] ESRD requires dialysis and is becoming a stumbling challenge to public health care systems due to the unaffordable cost of treatment of renal transplant in developing as well as in developed nations.[88]

It is caused by uncontrolled high blood sugar, and is characterized by an higher urine albumin excretion greater than 300mg in 24-hour collection in the absence of other renal diseases. [9, 94] Microalbuminuria is considered to be an early stage of DN. Microalbuminuria is also considered to be a predictor for CVD both among DM and non-DM subjects, and is one of the components of the metabolic syndrome. [93]

HISTORY OF DIABETIC NEPHROPATHY:
Before the advent of insulin therapy, DN was essentially an unknown entity, but as World War II loomed on the horizon, so did the beginnings of the equally devastating epidemic of DM ESRD, which we now face. [4] Bright, a physician from Guy’s hospital in 1836, gave the description of presence of albumin in
urine is related to some chronic kidney disease. This finding, along with the earlier observations by Cotunniusin in 1770 and Rolloin in 1798 that proteins are present in urine of some diabetic patients, led Rayerin in 1840 postulated that DM might develop a form of "Bright's disease". [95]

A British physician Clifford Wilson (1906-1997) and American physician Paul Kimmelstiel (1900-1970) in 1936 were the first to report this syndrome. [87] In the early 1980s, studies from Europe revealed that small amounts of albumin in the urine, not usually detected by conventional methods, were predictive of the later development of proteinuria in T1DM and T2DM patients. This stage of renal involvement was termed microalbuminuria or incipient nephropathy. [87, 96, 97]

**Epidemiology of Diabetic Nephropathy:**

DM-related deaths amounted to almost 3 million, equivalent to 5% of world’s all-cause mortality in 2000. [98] According to the World Health Organization (WHO), it is anticipated that the number of DM patients worldwide will grow to around 370 million by 2030. [99] In parallel with the increase in DM, a dramatic increase in the prevalence of DN has been noted, which has become commonest root of ESRD. [100]

DM is now the leading cause for ESRD worldwide, accounting for approximately 40% of patients receiving renal replacement therapy each year. Its prevalence is rising in parallel to that of DM. ESRD and dialysis treatment incur substantial cost in health, social and financial terms. [101] It has been reported that about 20-40% of T1DM & T2DM patients after 20-30 years after onset of DM develop DN. [99]

According to ethnicity there is variation seen in prevalence of DN: the higher prevalence is observed in African-Americans, Asians and Native-Americans. African-Brazilian population are more vulnerable to develop ESRD than the Europeans, but the prevalence of micro and macroalbuminuria did not differ. The prevalence of kidney damage in T1DM is higher than in T2DM in Caucasians. Although as the overall prevalence of T2DM is more than T1DM,
so kidney damage is more frequent in T2DM patients. The study conducted in Pima Indians in 1990, around 50% T2DM patients have had nephropathy after 20 years of onset of disease, and about 15% of them were already developed ESRD. [102]

The number of DN patients undergoing kidney replacement therapy increased twofold in the United States in the 1991-2001 period. The European Diabetes (EURODIAB) Prospective Complications Study Group and 18-year Danish study showed that the overall prevalence of microalbuminuria in T1DM and T2DM patients was 12.6% and 33%, respectively. According to the United Kingdom Prospective Diabetes Study (UKPDS), in Great Britain the annual incidence of microalbuminuria in patients with T2DM was 2% and the prevalence is 25% ten years after the diagnosis. [87]

Many countries have adopted renal registries, such as Finland, Hong Kong and the United Kingdom, and this shows that diabetic renal disease remain the single most common cause of renal failure, amounting to 24.8%. Epidemiology studies of T2DM patients show that DN prevalence ranges from 7.6% to 55%, while in different international registries it varies between 11.5% in United Kingdom to 42.9% in Thailand. [90]

In India, DN is expected to develop in 6.6 million of the 30 million patients suffering from DM. [94] In the elderly, DN today accounts for no less than 46% of CKD. In the "Chennai Urban Rural Epidemiology Study," the prevalence of overt nephropathy and microalbuminuria was 2.2% and 26.9%, respectively, in the urban citizens with DM. [100] Once DN is diagnosed, it increases the risk of development of other diabetic complications, including retinopathy affecting eyes and neuropathy affecting nerves, and affects the economic burden of individual. [9]

**Classification of Diabetic nephropathy:**

In the early 1980's, number of scientists reported the “preclinical” stage of DN characterized by excretion of albumin in urine but it is not detectable by standard laboratory methods and it was termed as ‘microalbuminuria’. DN has
been informatively categorized into 3 stages. The cutoff values of urinary albumin excretion rated used to define the stages of DN are described in Table 1. [95, 102]

**Table 1: Definitions of abnormal albumin excretion**

<table>
<thead>
<tr>
<th></th>
<th>Urinary albumin excretion rate (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal</strong></td>
<td>&lt;30</td>
</tr>
<tr>
<td><strong>Microalbuminuria</strong></td>
<td>30-300</td>
</tr>
<tr>
<td><strong>Macroalbuminuria</strong></td>
<td>&gt;300</td>
</tr>
</tbody>
</table>

The natural history of DN in T2DM is very similar to that in T1DM with the exception that small amount of albumin is already excreted in urine (microalbuminuria stage) at the time of diagnosis, reflecting the certainty that large number of T2DM patients have the onset of disease long before diagnosis. This leads to the series of events in the development of kidney changes in DM is identifiable. [95]

The revised Classification takes into account findings on the prognosis of T2DM patients from a ‘historical cohort study’ carried out as part of the Ministry of Health, Labor and Welfare-subsidized Project on Kidney Disease, entitled ‘Diabetic Nephropathy Research, from the Ministry of Health, Labor and Welfare of Japan.’ Patients with microalbuminuria are diagnosed as having incipient nephropathy. All patients with a GFR of less than 30 mL/min/1.73 m² are classified as showing kidney failure, regardless of their urinary albumin/protein values. [104]

**Stage I**: Early hypertrophy and hyperfunction stage is characterized by increase in renal plasma flow and GFR. This stage precedes about 5 years approximately after the onset of the disease. During this stage there is approximately 20% enlargement in kidney is seen and the blood flow to kidney is increased by 10%-15%, while albumin excretion rate and blood pressure remain unchanged.

**Stage II**: The quiet stage (silent stage), glomerular lesion without clinical
disease, which is associated with subtle morphological changes, including thickening of the GBM, glomerular hypertrophy, mesangial, and tubulointerstitial expansion. This stage is observed approximately two years after earlier stage. This stage shows no clinical signs of the condition. Most of the patients die before heading to the next stage of nephropathy.

Stage III: Incipient nephropathy/microalbuminuria, with likely onset of hypertension and urine albumin excretion of 30-300mg/day, termed as the microalbuminuria stage or incipient nephropathy. Clinically presence of this stage is the sign of glomerular damage. This stage appears at about 5 to 10 years after the start of the disease. Blood pressure remained normal or sometime hypertension is seen. Out of all patients near about 40% of patients end with this stage.

Stage IV: Chronic kidney failure (CKF) or overt DN/macroalbuminuria stage, characterized by dipstick positive proteinuria: urine albumin excretion > 300 mg/day. It is the irreversible stage. GFR decreases below 60 mL/min/1.73 m², and increase in the blood pressure is observed.

Stage V: ESRD with uremia: (GFR < 15 mL/min/1.73 m²). At this stage about 50% of the patients undergo peritoneal dialysis, blood transfusion, and the only permanent treatment is renal replacement therapy.

The hypertrophy and changes in the doppler indicators are the early morphological changes of kidney damage in the initial stages of DN, while degree of damage can be assessed by proteinuria and GFR. [87, 95, 104, 105]

Natural History of Diabetic Nephropathy:

It has been known that microalbuminuria indicates the risk of progression to overt albuminuria for more than a decade in both T1DM and T2DM. It is a warning sign for clinicians to prevent the progression of microalbuminuria to proteinuria. Various studies had reported decline in kidney functioning once the proteinuria occurs, irrespective of type of DM and therefore microalbuminuria is considered as indicator of DN progression. [95]
The excretion of albumin in normal individuals is about 10-15 mg/day. This amount of albumin cannot be detected by the standard laboratory tests, unless it is in excess of 300 mg/day, which is clearly defined as abnormal, even which is not detected by these tests. This rate of albumin excretion (i.e. 30-300 mg/day) was termed as microalbuminuria (incipient nephropathy) and is the earliest laboratory and clinical confirmation of DN. [9, 88, 95, 105]

It occurs in 30% of T1DM patients, [88] without specific interventions, 80% of individuals with T1DM who develop sustained microalbuminuria have their urinary albumin excretion increase at a rate of 10–20% per year to the stage of overt nephropathy or clinical albuminuria over a period of 10–15 years, with hypertension also developing along the way. The incidence of ESRD develops in T1DM patients is about 50% with overt nephropathy within 10 years and in 75% by 20 years. [9]

The incidence of microalbuminuria is found to be much higher in T2DM patients and may shortly lead to overt nephropathy after the diagnosis of DM, because the onset of DM is far before the diagnosis of disease is made. [9, 105] Without any medications, 20–40% of T2DM microalbuminuric patients develop proteinuria. Once proteinuria appears, renal function progressively declines and ESRD is reached after the next 7 to 10 years. [9, 88]

**Risk factors of Diabetic Nephropathy:**

The various longitudinal and cross sectional studies, identified number of risk factors in the pathogenesis of DN, including race, genetic predisposition, hypertension, hyperglycemia, hyperfiltration, smoking, and possibly, male gender, dyslipidemia, and age. [105] Other contributing risk factors are glomerular hyperfiltration, smoking, dyslipidemia, levels of proteinuria, and source of protein and fat in the diet. [106] They can be divided into non-modifiable (age, race, genetic factors) and modifiable (hyperglycemia, hypertension, dyslipidemia, and GFR) risk factors. [87]
Age:

In patients with T2DM, age and duration of DM are found to be positively associated with risk for albuminuria. In a study of Pima Indians in the population with T2DM, the subjects with early onset of DM before age 20 have had increased risk of developing ESRD. [87]

Race:

African Americans, Mexican Americans, Asian Indians, Pima Indians, and Hispanics compared with Caucasians have increased incidence and severity of DN. It is also higher in Blacks which is about 3 to 6 fold in comparison than Caucasians. [87, 105] This finding in genetically conflicting populations suggestive of socioeconomic factors playing the key role include diet, poor glycemic regulation, increased blood pressure, and body weight. [87] In blacks, the adjustment for confounding factors like lower socioeconomic status and higher incidence of hypertension, 4.8 times increased risk of development of ESRD was observed than the Caucasians. [105]

Genetic predisposition (Genetic risk factors):

Only about 10%-40% DM patients are prone to nephropathy, the epidemiological studies have revealed that genetic predisposition is an important factor in the development of DN in patients with both T1DM & T2DM. [4, 106] In studies of sibling-pairs, parent-offspring pairs or studies of extended families the increased aggregation of DN was observed. The chance of developing DN is increased 2-3 times if the sibling of affected has DN. [87, 102, and 105]

The exact genetic mechanisms in pathogenesis of DN development is not known, but studies reported few genes may interact with the environmental factors leading to DN. These genes may take part in the development of proteinuria, few are related to decrease in GFR and some genes are known to cause both the situations. The knowledge of these genes can identify the individuals susceptible to DN, therefore the preventive interventions can be
given to prolong the onset of DN thereby increasing the life expectancy. [102]

**Increased blood pressure (Hypertension):**

The prevalence of high blood pressure in DM population is 1.5 to 3 times more than that in non-diabetic population. [105] Increase in the arterial blood pressure is a key risk factor and known appropriate factor in development and/or progression of DN. The UKPDS study indicated every 10 mmHg fall in systolic blood pressure is associated with a 13% decrease in the risk of microvascular complications, with the lowest risk with normal blood pressure (<120 mmHg). One third of T2DM patients, already suffered hypertension at the time of diagnosis. [4, 87]

**Glomerular hyperfiltration:**

One of the important risk factor at diagnosis for development of DN in increased GFR. [87] Along with intraglomerular hypertension, glomerular hyperfiltration are the early physiological abnormalities, followed by the first clinical sign of renal damage in DM i.e. presence of microalbuminuria. [107] One third of the T2DM patients show the presence of elevated GFR values and this might be the causal factor for DN due to damage to glomeruli. [102] It changes structural and hemodynamics, with the resulting glomerular hyperfiltration and hypertrophy and damage to the endothelial wall. [87]

**Glycemic regulation:**

Of the risk factors for development of microalbuminuria uncontrolled glycemic status is a significant one, in either types of DM. It is reported that 37% decrease in endpoints in microvascular complication was seen when the HbA1c is reduced by 1%. The role of metabolism is not well defined in presence of micro- and macroalbuminuria, though it is shown to decrease GFR due to high glucose levels. Moreover, the renal damage was found to be reversed after pancreas transplantation in T1DM patients with mild to advanced DN lesions. [4, 102]
Overweight:

In patients with DM the risk of development of CKD increases with increase in body mass index (BMI). In these patients the decrease in urine protein excretion and improvement in kidney functioning has been noted with adequate diet and reduction in body weight. [87]

Smoking:

Smoking might contribute to progression of DN. Although some studies did not confirm these observations, it is strongly recommended to quit smoking in any phase of DN, also aiming to reduce the associated cardiovascular and cancer risk. [102]

Dyslipidemia:

In T1DM patients increased serum triglycerides, total and LDL-C were associated with micro- and macroalbuminuria. [102]

Proteinuria:

Proteinuria as it is a characteristic of DN, but may play a role in progression of DN. Higher risk of ESRD has been found to be associated if protein excretion is >2 g/24 hours. Increased excretion of albumin through kidney may also initiate damage to glomeruli may be via activation of inflammatory pathways. This is one of the strategy to focus on decreased urinary albumin excretion in treatment of DN patients. [102]

Dietary factors:

In patients with T1DM increased intake of proteins is found to be positively associated with the high albumin excretion rate, while this association was not documented in T2DM patients. Along with high protein intake the protein source also an important factor for presence of DN. In T1DM patients, the higher fish protein intake is known to decrease the risk of microalbuminuria, via the unknown mechanisms but this might be associated with hemodynamic factors. [102]
PATHOPHYSIOLOGY OF DIABETIC NEPHROPATHY

DN refers to a characteristic set of structural and functional kidney abnormalities in patients with DM. [105] The pathogenesis of DN has been intensely investigated, and the roles of various mechanisms have been established. [108] Pathology of DN is very vast, and interaction of hemodynamic (systemic and glomerular hypertension) and metabolic factors (hyperglycemia and possibly hyperlipidemia) are the important factors for it. The other factors under investigation include endothelial dysfunction, inflammation and oxidative stress. Early hemodynamic changes like glomerular hyperperfusion, hyperfiltration, and increased intraglomerular pressure lead to subsequent proteinuria, systemic hypertension, GBM thickening, glomerular hypertrophy, nodular and diffuse glomerulosclerosis, tubular atrophy, interstitial fibrosis, mesangial cell expansion, podocyte injury and eventual loss of renal function. [87, 99, 106, 107]

Uncontrolled blood glucose and high blood pressure are the most vital factors in the development and progression of nephropathy. [95] The humoral mediators, cytokines and growth factors are secreted due to hyperglycemia by the resident and nonresident renal cells, and these factors have a role in structural changes such as increased deposition of extracellular matrix and functional changes such as increased permeability of GBM, contributing in the development of DN. [99]

In a susceptible patient, hyperglycemia appears to initiate nephropathy through the enhanced generation of ROS and increased formation of AGEs, up regulation of transforming growth factor-beta 1 (TGF-β1), PKC activation, activation of polyol and aldose reductase pathway (Figure 4). Both ROS and AGEs contribute to glomerular cell injury and the development of microalbuminuria, which may signify systemic alterations in endothelial function in capillary beds and also in large vessels. [4, 99, 107, 108] The oxidant which is known for prevention of flow mediated dilation of blood vessels, “nitric oxide” is consumed by oxidative stress and this leads to injury to the endothelium. This is followed by secretion of cytokines, speeding up of
inflammation and worsens the rigidity of blood vessels due to atherosclerosis. [107]

DN is characterized by excessive, gradual and progressive deposition of extracellular matrix in the kidney. With renal damage, there is pathological change in mesangial and vascular cells, leading to glomerular mesangial expansion, GBM, nodular and diffuse glomerulosclerosis and tubulointerstitial fibrosis. [99, 105, 109] These changes lead to various cellular responses, expression of secretory factors and extracellular matrices that ultimately result in development of DN. [108] Schematic illustration of the interaction between hemodynamic and metabolic factors in the pathophysiology of DN is depicted in figure 5.

The inflammatory pathways are activated by entry of macromolecules through GBM, which is a secondary cause of kidney damage. [107] Later the characteristic lesion of the Kimmelstiel–Wilson nephropathy, the mesangial nodules are formed and represents with additional extensive tubule-interstitial lesions. [95]

**Heparanase Expression:**

The mechanism of heparanase synthesis regulation is also has principal role in the pathogenesis of DN. The negative charge of glycocalyx is change due to the reduced synthesis of heparin sulfate on the surface of endothelial cell, this as a result increases permeability and filtration of albumin in the glomerular membrane. [87]

**Prorenin:**

The precursor of renin, ‘prorenin’ in children and adolescents play a role in the pathogenesis of DN. The binding of prorenin to its specific receptors, activates the mitogen-activating protein kinases (MAPK) signaling pathway, which enhances the risk of development of renal damage. [87]
Figure 5: Schematic illustration of the interaction between hemodynamic and metabolic factors in the pathophysiology of DN. [110]

SCREENING FOR ALBUMINURIA:

Current guidelines recommend controlling blood glucose, blood pressure, and lipid levels, smoking cessation, and administration of antiplatelet agents in order to delay the progression of DM complications. Annual testing of serum creatinine and urine albumin excretion is recommended to screen for
nephropathy. However, beyond recommendations for screening and treatment, there is no practical method to predict the patients likely to progress to end points such as ESRD. [109]

For all T2DM patients at the time of diagnosis of disease the test for presence of microalbumin should be performed. Microalbuminuria rarely occurs with short duration of T1DM; therefore, screening in individuals with T1DM should begin after 5 years’ disease duration. Because of the difficulty in precise dating of the onset of T2DM, such screening should begin at the time of diagnosis. After the initial screening and in the absence of previously demonstrated microalbuminuria, a test for the presence of microalbumin should be performed annually. Screening for microalbuminuria can be performed by three methods: [9]

1. Measurement of albumin-to-creatinine ratio (ACR) in a random spot collection; [9] for the screening of microalbuminuria in DM patients ACR should be performed on a yearly basis. The test of ACR should be repeated, if the value is between 3.0 mg/mmol and 70 mg/mmol. The elevated ACR results for two or more occasions confirms microalbuminuria; however, and if ACR value initially is ≥70 mg/mmol, repeat sample is not required. [107]

2. Urine creatinine measurement in 24 hours urine samples, allows the measurement of creatinine clearance giving idea about GFR; [9] and

3. Timed (e.g., 4 hours or overnight) collection: Microalbuminuria is said to be present if urinary albumin excretion is 30 mg/24h. Short-term hyperglycemia, exercise, urinary tract infections, marked hypertension, heart failure, and acute febrile illness can cause transient elevations in urinary albumin excretion. [9]

Before processing the urine samples for above mentioned tests, possibility of urinary tract infection (UTI) should be excluded, as false positive results are observed due to UTI. [107]
TREATMENT:

Indians have highest prevalence of DM in world and an excess of avoidable complications and early death. Many of these complications can be reduced with appropriate community-based primary health care interventions. [94]

The optimal therapy for DN is prevention. As a part of comprehensive DM care, microalbuminuria should be detected at an early stage when effective therapies can be instituted. Interventions effective in slowing progression from microalbuminuria to overt nephropathy include: (1) strict glycemic control, (2) strict blood pressure control, (3) administration of ACE inhibitors or ARBs, (4) treatment of dyslipidemia, and (5) restriction of protein intake. Patients who develop ESRD will require renal replacement therapy. [105, 111]

Strict Glycemic Control

The stage of DM at which glycemic control was started and consequent glucose metabolism normalization are known to contribute to the overall effectiveness of strict glycemic control. [100] Improved glycemic control reduces the rate at which microalbuminuria appears and progresses in T1DM and T2DM. However, once overt nephropathy exists, it is unclear whether improved glycemic control will slow progression of renal disease. During the phase of declining renal function, insulin requirements may fall as the kidney is a site of insulin degradation. Furthermore, glucose-lowering medications (sulfonylureas and metformin) are contraindicated in advanced renal insufficiency. [111] Strict glycemic control along with use of insulin during microalbuminuria in DM patients was found to prolong the onset of overt nephropathy. [95]

Strict Blood Pressure Control

Many individuals with T1DM or T2DM develop hypertension. The progression of DN and other complications in T2DM patients was prevented by strict blood pressure control. It is demonstrated that strict blood pressure control reduces albumin excretion and slows the decline in renal function. [111]

The UKPDS study reported for every 10 mmHg reduction in blood pressure
decreases the risk of DM complications by 13%; when the systolic blood pressure is below 120 mmHg, the risk is minimal. Current guidelines for treatment of arterial hypertension, in DM patients the blood pressure should be maintained <130/80 mmHg. Even if the blood pressure values are in upper normal range, antihypertensive treatment is recommended. [87, 100]

**Inhibition of RAS**

ACE inhibitors and ARBs reduce the progression of overt nephropathy in individuals with T1DM or T2DM. It should be prescribed in individuals with T2DM or T2DM that may prevent microalbuminuria, an indicator of early kidney injury and risk of cardiovascular disease as well in patients with DM. After 2 to 3 months of therapy, measurement of proteinuria should be repeated and the drug dose increased until either the albuminuria disappears or the maximum dose is reached. ARBs can be used as an alternative in patients who develop ACE inhibitor-associated cough. Both ACE inhibitors and ARBs can induce hyperkalemia or renal insufficiency. If use of either of these types of agents is not possible, then calcium channel blockers (non-dihydropyridine class) can be used. However, their efficacy in slowing down the fall in GFR is not proven. [87, 100,111]

**Dyslipidemia**

The lipid derangements are common in patients of DM, and its get worsened as the patient develops DN. The abnormal lipid profile increases the risk of CVDs, therefore the reduction of lipids in the plasma is an important therapeutic intervention for e.g. use of statins, which are known to slower the progression of DN. Administration of fenofibrate in DM patients decreases albumin excretion rate. Apart from its anti-inflammatory action, it diminishes collagen type-1 synthesis in mesangial cells via nuclear PPAR-α receptors. Use of RAS inhibitors with good glycemic regulation, keeps the blood pressure in control, this is the optimum therapeutic strategy along with lipid lowering drugs in patients with DM and DN. [87, 100]
The Role of Other Factors

Transforming growth factor beta (TGF-β) has been shown to have a protective effect against DN in experimental DM models. [87, 100] Synthesis of proteins by adipose tissue, lipid metabolism, insulin action, inflammation, and control of blood pressure are well regulated by mediation of peroxisome proliferator-activated receptors (PPAR); however, they also have an important role in development of DN in T2DM patients. Use of PPAR-γ agonists, like thiazolidinediones, reduce excretion of albumin in various DN stages by maintaining normal blood pressure, in addition to reduction of fibrosis, mesangial proliferation, and inflammation. [87, 100]

New Treatment Strategies

Current approach of treatment for DN patients is not successful for all patients, therefore, new strategies for treatment of DN needs to be studied. In animal models use of higher doses of vitamin thiamine has been shown to decrease the rate of development of microalbuminuria, by altering the PKC activation, glycation of protein, and lowering oxidative stress. ALT-711 use in animal studies metabolizes AGEs, decreases blood pressure and renal damage. Ruboxidtaurin a PKC-β inhibitor regulates normal GFR, lowers albumin excretion, and also renal functioning improvement was observed. Pimagedine, the next generation AGE inhibitor also have similar effects on GFR and albumin excretion rate in T1DM patients and proteinuria. In an experimental model of induced glomerulosclerosis, modified heparin glycosaminoglycan prevented albuminuria, accumulation of extracellular matrix proteins, and increased expression of TGF-beta. [87, 100]

LIPID PROFILE IN DIABETIC NEPHROPAHTY:

The incidence of DM is increasing rapidly and studies have suggested that an abnormal lipid profile in DM may lead to worsening the condition and direct the disease to renal impairment. Elevated lipoproteins and lipids are one of the prime reasons for injury to glomeruli and tubulointerstitial cells in DM contributing to the advancement of DN. However, use of lipid lowering drugs in
these patients reduces rate of albuminuria. Abnormal lipid profile also results in cardiovascular morbidity and mortality associated with DN. [106]

In DM patients, the increased glycation of proteins causes stiffness of arteries, and thereby progression to arteriosclerosis. This lowers the elasticity and may cause hypertension in DM patients which is an independent risk factor of mortality. [87]

In addition to its being the earliest manifestation of nephropathy, albuminuria is a marker of greatly increased cardiovascular morbidity and mortality for patients with either T1DM or T2DM. Therefore, measurement of microalbuminuria can provide the possibility of CVDs, so the patient will receive aggressive intervention to reduce CVD risk factors. [9] In T1DM patients, the increased intake of saturated fats from diet potentiates the development of microalbuminuria, and decreased consumption of polyunsaturated fatty acids increases the risk of microalbuminuria in T2DM patients. [102]

Many observational studies suggest that lipids may play a role in the development and progression of glomerular injury. It is found that the level of cholesterol both at onset and after a five-year follow up period was positively related with the subsequent increase in urinary albumin excretion in microalbuminuric patients with T2DM. And albumin excretion in urine was also significantly related to the level of total serum cholesterol. In T1DM, decrease in kidney functioning was correlated with increased total cholesterol and decreased HDL-C. [105] Few studies had suggested to lower the risk of proteinuria by decreasing the level of cholesterol. [9]

Various approaches for treatment of DN in T1DM patients like controlling hyperglycemic state, lowering protein intake, and maintaining normal blood pressure may slow down the development of nephropathy. But as almost 45% of T2DM patients at the time of diagnosis have abnormally increased GFR compared to non-diabetics as well as overweights. These patients are more prone to development of atherosclerosis and also alter GFR as well as size of the
glomeruli. [87]

**Katore [13] et al.** (2014) assessed atherogenic lipid profile in patients of DM and DN. Values of TC, TG, LDL-C and TC/HDL, LDL/HDL ratios were significantly higher in DM with nephropathy and DM without nephropathy but values of HDL-C were significantly lower in DM with nephropathy and DM without nephropathy as compared to controls.

**Suchitra [112] et al.** (2013) studied atherogenic dyslipidemia in T2DM and DN patients. Atherogenic dyslipidemia with elevated Lp(a), TC, TG, VLDL, LDL, non-HDL-C, lipid ratios, atherogenic index and low HDL levels were observed in T2DM patients with or without nephropathy when compared to controls. Significantly high TG/HDL, TC/HDL and atherogenic index were observed in DN when compared to T2DM.

**Momin [113] et al.** (2014) found significant increase in the lipid and lipoprotein levels, except HDL-C which were significantly decreased in T2DM than controls. BMI, FBG, TC, TG, LDL-C, VLDL-C, TC/HDL-C, TG/HDL-C, LDL-C/HDL-C, Non HDL-C and cholesterol retention fraction were positively associated with insulin level and HOMA IR, while HDL-C was negatively associated with insulin and insulin resistance.

**Khadke [114] et al.** (2015) undertook the study to assess the lipid profile of T2DM and its correlation with FBG. The lower levels of HDL-C and LDL-C were reported in DM patients, and in patients taking statins and had low LDL-C. The significant difference in FBG, lipids and risk ratios was indicated in a correlation analysis. The correlation of alterations in lipid levels with FBG were the key findings in diabetics, along with the increased risk of CVDs.

**Samatha [115] et al.** (2011) investigated the predictive biomarkers of microvascular complications in T2DM patients. The incidence and the progression of microvascular complications were found to be increased with hyperglycemia, a longer duration of DM, dyslipidemia and the extent of microalbuminuria in patients with T2DM. Therefore, poor glycemic control, a longer duration of DM, dyslipidemia and the progression of microalbuminuria
can predict the microvascular complications in patients with T2DM.

**Ozder [116]** (2014) found the association between serum levels of TC, TG, LDL and hepatosteatosis and HbA1c. Significantly higher levels of TC, TG and LDL, and lower HDL-C were noted in patients with DM. FBG showed significant positive correlation with TC and TG.

**Sigdel [117] et al.** (2008) gave the prevalence of microalbuminuria and overt proteinuria to be 45.5% and 11.2%, respectively. Prevalence of microalbuminuria in females was found marginally higher than in males. Also, microalbuminuric patients had significantly higher blood pressure.

**Marcovecchio [118] et al.** (2009) explored the prevalence of lipid abnormalities and their relationship with albumin excretion and microalbuminuria in adolescents with T1DM. TC, LDL-C, HDL-C, and non-HDL-C were found to be higher in females than in males. A1C was found independently related to all parameters except HDL-C. Total cholesterol and non-HDL-C were independently related to longitudinal changes in ACR.
ANGIOTENSIN CONVERTING ENZYME:

T2DM and DN are chronic progressive disorders that are related to a group of genetic, lifestyle and environmental factors. [119] DN, an important microvascular complication of T2DM and is the major cause of ESRD observed in about 30% of these cases.[33, 34] South Asian T2DM patients have been shown to have a higher prevalence of nephropathy when compared to Europeans. [120]

The exact etiology is unknown, but possible factors triggering the DN include chronic hyperglycemia, systemic and intra-renal hypertension, dyslipidemia, smoking, obesity, aging, high degree of insulin resistance, male gender, race, possibly high dietary protein intake, familial clustering of DN and genetic polymorphisms. [121] Some T2DM patients with excellent blood glucose control and even after being under intensive insulin therapy, progress towards DN, while most patients with long standing severe hyperglycemia and antihypertensive therapy never develop. [33] This complexity adds to the uncertainty about predisposition of T2DM patients to complications like nephropathy and all diabetic patients are seen at equal risk of developing DN and assigned aggressive glucose control therapy (Add Reference).

Clinical disparity in extent of glycemic control and appearance of DN in diabetic patients indicates that factors other than chronic hyperglycemia may equally contribute to susceptibility to DN. Researchers have sought the answer to this dilemma at the genetic background of the patients. [119, 122]

Various genes have been mapped at the loci susceptible to DN, this finding realized the role of genetic factors in the development of this microvascular complication. [34] Several studies of DN have analyzed the candidate genes previously studied in hypertension, T2DM, and CVD. [122] Polymorphisms in genes encoding PPAR-γ, eNOS, GLUT-1, aldose reductase, MTHFR, apo-E and components of the RAS including angiotensinogen, Ang-II receptor type 1, and particularly, the ACE gene, have been implicated in the pathogenesis of DN. [121]
Figure 6: Mechanism of RAS system [124]
In experimental and human DN, systemic and glomerular hypertension plays a role in its initiation and progression. These hemodynamic changes may be explained in part by alterations in the RAS. Consequently, genes encoding elements of RAS have been advocated as potential genetic predispositions for the development of hypertension and CVD, which are common in individuals with DN. Angiotensin-I converting enzyme (ACE) is one of the key enzymes in the RAS. [32, 119] This system regulates blood pressure, constriction of vessels, and release of ADH, also electrolyte balance. [123] The overview of renin-angiotensin-aldosterone system is illustrated in figure 6.

The gene for angiotensin I– converting enzyme (ACE) was tested for its association with DN, because of high intraglomerular angiotensin II levels cause intraglomerular hydraulic pressure to increase, which favors diabetic glomerulosclerosis, and low ACE concentrations can limit intrarenal Angiotensinogen II generation. [125] Angiotensin-converting enzyme (ACE, EC 3.4.15.1) is an exopeptidase which mediates various physiological functions. [122]

It is one of the first candidate genes studied in DN for several reasons, [126] like activation of vasoconstrictor peptide angiotensin to maintain normal blood pressure and on other hand it inhibits the action of bradykinin a vasodilator peptide. By activation of angiotensin II it also regulates microcirculation in the kidney. [33]

**Structure and functions of ACE:**

Angiotensin I, a decapeptide generated by action of the enzyme renin on a glycoprotein substrate angiotensinogen, is converted to the vasopressor octapeptide, angiotensin II. The exopeptidase responsible for this conversion was first identified and isolated in plasma by Skeggs et al., [127, 128] who named it angiotensin converting enzyme. This enzyme is a halide-activated, EDTA-sensitive, peptidase that catalyzes the cleavage of dipeptidyl residues from the -COOH termini of peptide substrates, and releases His-Leu from the -COOH terminus of the decapeptide angiotensin I. [129] Crystal structure of
human testicular ACE with the inhibitor is shown in figure 7.

![Crystal structure of human testicular ACE](image)

**Figure 7:** Crystal structure of human testicular ACE with the inhibitor (lisinopril) molecule bound at the centre of the molecule. The green sphere represents the zinc ion and the red spheres represent the bound chloride ions. [130]

The gene encoding ACE is found on chromosome 17q23 which modulates ACE level. ACE genes have been cloned from human, mouse, and rabbit. The human gene has 21kb long containing 26 exons and 25 introns (Figure 8). [33, 34]

![Gene structure of the ACE gene](image)

**Figure 8:** Gene structure of the ACE gene consisting of 26 exons and 25 introns. The I/D polymorphism (rs1799752) affects intron 16. [133]
Out of 26 exons, exons from 1 to 12 code for the N terminal domain and remaining exons from 13 to 26 code for C terminal domain. The ACE, which was originally discovered in equine plasma, is a membrane-bound dipeptidyl carboxypeptidase ecto-enzyme located in the endothelial lining of blood vessels throughout the body. The ACE is found as a membrane-bound enzyme in endothelial cells and different types of epithelial and neuroepithelial cells as well as in circulating form in biological fluids, such as plasma, cerebrospinal fluid, amniotic fluid, and seminal fluids.

ACE catalyzes conversion of a potent vasoconstrictor angiotensin-I to angiotensin-II, angiotensinogen II increases systemic and glomerular blood pressure, up regulates mesangial cell proliferation and tissue growth. Through this conversion it plays an important role in proliferation of vascular smooth muscle cells. The action of ACE in renin angiotensin system is depicted in figure 9.

![Figure 9: The functions of ACE in renin–angiotensin system (RAS).](image-url)
It is also known to mediate regulation of blood volume, arterial pressure, cardiac and vascular function, and electrolyte metabolism. [121]

**ACE I/D Polymorphism:**

Nearly 160 gene polymorphisms in ACE gene are found and many of them are single nucleotide variations. [33] It was reported first that the ACE insertion/deletion (I/D) polymorphism has the presence (I) or absence (D) of a 287 bp DNA sequence situated in intron number 16 of the ACE gene. It is the commonest and extensively studied polymorphisms in DN. [33, 122] Since 1990, ACE I/D polymorphism association with DN has been extensively investigated and more than 300 studies have explored genetic associations of this polymorphism in more than 100 conditions including DN. [32] The ACE I/D polymorphism in 16th intron of this gene results in 3 genotypes viz. DD & II homozygotes and ID heterozygotes. [34, 123]

As the polymorphism is found in an intron, it does not change the structure of the enzyme. [119] The ACE (I/D) polymorphism has been suggested to be a significant risk factor for various CKD, coronary artery disease, coronary heart disease (CHD) and hypertension. The increased risk of DN was shown to be associated with presence of DD genotype. [123] The angiotensin-converting enzyme (ACE) gene I/D genotype is associated with plasma, cellular, and tissue ACE levels. [134] The ACE levels have been shown to be genetically controlled. [126] The physiologically decreased ACE activity is associated with this polymorphism of ACE. [33]

This polymorphism accounts for over 40% of interindividual variability of serum or tissue ACE activity. [32] It accounts for differences in plasma ACE levels and was thought to influence ACE-mediated physiological functions, and for its genetic susceptibility to glomerular lesions. [121, 122, 125] Several studies have found the D allele to be an independent risk factor for DN and it is used as a marker in population structure analyses. Plasma ACE levels are the highest in subjects with the DD genotype, intermediate in subjects with the ID genotype, and the lowest in subjects with the II genotype. [134] Several
Japanese studies have found the D allele to be an independent risk factor for DN. [120] The various physiological functions to increase the oxidative stress by angiotensin II is shown in figure 10.

![Diagram showing vasopressor mechanisms in renovascular hypertension](image)

**Figure 10: Schematic of vasopressor mechanisms identified in renovascular hypertension. [132]**

The role of I/D polymorphism of ACE has been studied in various ethnic groups with inconsistency in results. Ethnicity, interindividual variation determining factor and also defines the action of I/D polymorphism of ACE gene in susceptibility to DN. In Caucasians ACE D allele was not found to be associated with DN. However, the association of D allele of ACE and DN has been reported in Asian and French population. The higher enzyme activity of ACE and level of albuminuria are associated with presence of D allele in T2DM patients from Tehran. The D allele as well as DD genotype frequencies were higher in DN than DM patients without nephropathy in Asian Indian and Tunisian patients. [33]
The association of higher GFR and ACE levels also associated with DD genotype of ACE in T2DM patients, and consequently higher risk of DN development, increased protein excretion, more likelihood of progression of renal damage, and an addition to mortality due to dialysis. However, the presence of I and/or D allele of ACE gene is not a good predictor in Caucasians. [33, 105, and 126]

The increased activity of ACE in plasma and in tissues like heart and kidney, is associated with DD genotype than ID and II genotypes. This increased level of ACE leads to the pathogenesis of DN, may be due to increased activation of angiotensin II, increasing GFR and intraglomerular blood pressure, this promotes mesangial cell & matrix proliferation. [123]

Patel [123] et al. (2011) from eastern Indian population analyzed the ACE I/D gene polymorphism in T2DM with no complication, DN, non-DN patients and controls. No any role of ACE I/D polymorphism in the development of DM and non-DN was indicated, while DD genotype was found to be associated with development of DN.

Hussein [119] et al. (2015) conducted a case control study to investigate the possible role of ACE gene in the pathogenesis of nephropathy in patients with DM. In dominant and recessive models, they reported significant association of dominant model with the risk of DN which was found to be raised by three folds. The minor allele frequency (D) was significantly higher in DN when compared with that of the normal control group.

Khan [34] et al. (2011) in T2DM with nephropathy patients investigated I/D polymorphism of the ACE gene. The results indicated that T2DM patients with presence of D allele had almost 2 times higher risk of developing DN.

Marre [125] et al. (1999) tested the possible interaction between ACE I/D polymorphism and uncontrolled T1DM by measuring GFR and effective renal plasma flow (ERPF) during normoglycemia and hyperglycemia in 9 normoalbuminuric, normotensive T1DM subjects. T1DM individuals with the II genotype were found to be resistant to glomerular changes induced by
hyperglycemia, providing a basis for their reduced risk of nephropathy.

**Hadjadj [126] et al.** (2001) attempted to investigate the association of M235T polymorphism in angiotensinogen and ACE I/D polymorphism. The angiotensinogen M235T polymorphism was not found to be associated with renal events. The D allele of the ACE I/D polymorphism was shown to be a risk factor for both the onset & the progression of DN in T1DM patients. [126]

**Viswanathan [120] et al.** (2001) studied the association of ACE gene polymorphism and DN in South Indian subjects. They showed positive association between D allele (ID + DD) of the ACE polymorphism and diabetic proteinuria in South Indian T2DM patients.

**Mizuiri [134] et al.** (2001) investigated a possible association between the ACE I/D genotype and renal ACE mRNA levels in healthy subjects. It is suggested that renal ACE gene expression is associated with the ACE I/D genotype in healthy Japanese subjects.

**Arfa [135] et al.** (2008) investigated the association of the ACE I/D polymorphism with DN and T2DM in the Tunisian population. The results showed no association of I/D polymorphism within the ACE gene with DN nor with T2DM in the study population.

**Jayapalan [121] et al.** (2010) evaluated the genetic susceptibility of the ACE gene to DN in the multiethnic Malaysian population. The I/D polymorphism of the ACE gene was not found to be significantly associated with both T2DM and/or DN in this population regardless of ethnicity and gender.

**Bhaskar [122] et al.** (2013) carried out the study, to find out the role of ACE ID and PPARG P12A polymorphisms in genetic susceptibility of DN in south Indian population. No significant differences in genotype frequencies of ACE ID as well as PPARG P12A polymorphisms were found when DN patients were compared with controls. Also the synergistic role of ACE ID PPARG P12A interaction, did not show any association in patients with DN when compared to DM controls.
PODOCYTES:

In United States, DN commonly known to cause ESRD, and the similar picture is seen in developing nations as well. [33, 136] The complex mechanisms in the pathogenesis of DN are not elucidated completely. [33] The glomerular filtration barrier has three important components: endothelial cells, the GBM and the podocytes. [137]

Pathogenically and prognostically the renal podocyte injury is important in progression of DN. [33] The inflammatory reaction which is initiated due to the protein accumulation in the cytoplasm of proximal tubular cells causes formation of lesions in tubulointerstitial cells, and contribute in increasing proteinuria and developing glomerulosclerosis. [95]

Human podocytes (Pods) have been demonstrated to be functionally and structurally injured in the natural history of DN. Furthermore, the number and density of Pods have been reported to be markedly reduced (podocytopenia) in patients with DM. Pods are located outside the GBM. [136]

Recently, the role of podocytes in DN has become the subject of intense research effort since the early key events in DN include loss of podocytes in glomeruli. [108, 137] Subsequent analysis showed that the decrease of podocyte number is a good predictor of progression of albuminuria. [108] In the early stages of DN in T1DM and T2DM patients, the possible podocyte injury mechanisms include foot process effacement, tissue enlargement, detachment, apoptosis, and may be epithelial to mesenchymal transition (EMT). [33, 137]

The correlation of proteinuria has been evidenced with proteins or genes associated with podocyte. This increases the possibility of studying the podocyte associated proteins in progression of DN. [33] Number of genes expressed by podocytes have been identified and studied their role in the development of proteinuria and glomerular disease. [138] Because of the proximity of the apical region of Pods to the urinary space, pathological events occurring in this region are expected to be more easily detectable in urine and blood than those occurring in the basal or slit diaphragm regions of Pods. [136]
Structure and Functions of Podocytes:

The Bowman's capsule (figure 11) filters the blood, retaining large molecules such as proteins while smaller molecules such as water, salts, and sugars are filtered as the first step in the formation of urine.[139]

Podocytes are terminally differentiated, highly specialized epithelial cells with a cell body, and they play a critical role providing physical support, secretion of cytokines and in the glomerular function regulation. [33, 95, 108, 139] These cells have a prominent cell body contains the nucleus, and distinct, long processes, called foot processes, foot projections (or pedicels, for which the cells are named podo + cyte). [138, 140]

![Figure 11: Juxtaglomerular Apparatus and Glomerulus [141]]

These processes divide successively until the terminal foot process rests on the GBM. The structural support via the foot processes is provided by the podocyte 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. [95] They have microtubule-based thick primary foot processes and fine
actin-based secondary foot processes, which adhere to GBMs and interdigitate each other (figure 12). [108] Both decreased number of podocytes and foot process effacement, termed podocytopathy, has been reported to be related with DN. [33]

![Figure 12: Podocytes. In (a), the large cell body can be seen at the top right corner, with branches extending from the cell body. The smallest finger-like extensions are the pedicels. (b) This capillary has three podocytes wrapped around it. [141] The foot processes from the neighboring podocytes are connected with each other by a specialized intercellular junction, called a slit diaphragm. [108] Blood is filtered through these slits, and nephrin, a protein of slit diaphragm are essential to prevent filtration of proteins into Bowman’s space. [95, 140] The excretion of anionic proteins such as albumin are repelled by negatively charged molecules covering podocyte, like the basement membrane. It also keeps the slit diaphragm open and helps in bridging the gap between adjacent foot processes. [95]
Pathways of podocyte injuries: [138]

The research has not only clarified the mechanisms of podocyte injury as a major cause of proteinuria, but also un-enveloped this new knowledge and integrating it in new classification schemes where morphology, etiology, and mechanisms of podocyte injury are the variables that define each glomerulopathy.

Numerous pathways are known causing alteration in podocyte phenotype and the their involvement in the activity of different cellular apparati. These mechanisms are stimulated by number of etiologic factors. Podocytes can react with various stimuli translating into following 4 structural patterns of damage to glomeruli. (Figure 13):

1. **Effacement:** With number of proteins, injured podocytes by simply effacement with reorganization or redistribution of the cytoskeleton. This is seen in minimal change nephropathy.

2. **Apoptosis:** Podocyte detachment and death is encountered by engagement of fatal pathways. The glomerulus is perpetrated to sclerosis focal segmental glomerulosclerosis, once podocytopenia is detected.

3. **Arrested development:** With preserved proliferative activity, podocytes remain immature, typical stages of nephrogenesis. Immature appearance of glomeruli with increased mesangial matrix diffuse mesangial sclerosis.

4. **Dedifferentiation:** Injured podocytes by re-engaging the cell cycle, revert to a more immature state; this event is associated with morphologic changes in glomeruli causing glomerulopathy.
Proteinuria and progression of DN has been shown to be linked to reduction in podocyte number and density. Clinical and experimental studies have documented relationship between podocyte injury/loss and glomerulosclerosis, and documented that podocyte injury, involving podocyte hypertrophy and effacement is related with the onset of albuminuria. The mechanisms of injury in DN include glomerular hyperfiltration, toxicity of ROS and AGEs, increased action of angiotensin II, and increased synthesis of cytokines and growth factors. One of the possible mechanism of podocyte injury by increased activation of renin angiotensin system is outlined in figure 14.
Gene expression of podocyte proteins:

Currently, renal pathological examination, the gold standard method for evaluation of podocyte injury in DN patients, is inappropriate for physicians to closely monitor patients, due to the invasive nature of renal biopsy. Real-time PCR, a molecular technique is having excellent sensitivity, quantification, and reproducibility, and can even major very low amounts of genes from one single cell. Now a days very sensitive methods are available to extract the RNAs from...
urinary sediment, and RT-PCR, is applied to quantify expression of mRNA in it, and it appears to be recent modality for studying renal damage. The determination of mRNA levels may be useful for monitoring the renal disease progression. [33]

The understanding of proteinuria and its pathogenesis in DN patients has been increased by analyzing the expression of podocyte proteins. Nephrin and podocin are closely linked to cytoskeletal alpha actinin-4 and synaptopodin, enabling dynamic rearrangements of the podocyte architecture. Podocalyxin, a phenotypic marker of the podocyte at the apical membrane, limits the passage of negatively charged albumin. [39]

It should, therefore, be clinically useful, if podocyte loss could be monitored by measuring podocyte products in urine. [143] In albuminuria the podocytes are shedded in urine and it is an important contributor. Therefore, the loss of these podocytes directly supports its role in the development of albuminuria. A sequence of events through epithelial-mesenchymal transition and apoptosis or detachment, and ultimately contributing to glomerulosclerosis and decline of renal function, which might trigger the podocyte injury. [33]

Levels of urinary podocalyxin were elevated in patients with various glomerular diseases and patients with DM. In patients with DM, urinary podocalyxin was higher than the cut-off value in 53.8% patients at the normoalbuminuric stage, 64.7% at the microalbuminuric stage and 66.7% at the macroalbuminuric stage. Positive correlations were observed between urinary podocalyxin levels and HbA1c, urinary β2 microglobulin, α1 microglobulin and urinary N-acetyl-β-D-glucosaminidase, although urinary podocalyxin levels were not correlated with other clinical markers such as blood pressure, lipid levels, serum creatinine, estimated GFR or proteinuria. [136]
Aaltonen [144] et al. (2001) assessed the role of nephrin in two widely used animal models of DM, the streptozotocin model of the rat and the non-obese diabetic mouse. In both models, the expression levels of nephrin-specific mRNA increased up to two-fold during several weeks of follow-up. They concluded that nephrin is connected to the early changes of DN and thus may contribute to the loss of glomerular function.

Toyoda [145] et al. (2004) examined the expression of nephrin mRNA in the kidneys of T2DM with DN. Their results suggest that low expression of nephrin mRNA may be closely linked to development and/or progression of proteinuria in human DN.

Baelde [146] et al. (2004) evaluated mRNA expression profiles of from glomeruli of DM and healthy individuals. Oligonucleotide microarray analyses of control and diabetic glomeruli in relation to vascular damage, mesangial matrix expansion, proliferation, and proteinuria suggested that progression of DN might result from diminished tissue repair capability.

Wang [147] et al. (2007) measured the mRNA expression of nephrin, podocin, synaptopodin, Wilms’ tumor-1 (WT-1) and α-actinin-4 in urinary sediment. Urinary mRNA expressions of nephrin, podocin, synaptopodin, WT-1 and α-actinin-4 are higher in patients with DN than in normal controls. Urinary nephrin and synaptopodin expressions were correlated with baseline clinical parameters such as proteinuria or renal function, while WT-1 expression was found to be related to the degree of histological damage.

Wang [148] et al. (2010) measured the intra-renal and urinary mRNA expression of nephrin, podocin, and synaptopodin. Intra-renal expression of podocyte-associated molecules correlated with glomerular podocyte number, renal function, and tubulointerstitial scarring. The results suggest that intra-renal, but not urinary expression of podocyte-associated molecules might be used to assess the degree of podocyte loss in DN.

Zheng [33] et al. (2011) investigated excretion rate of mRNAs of podocyte
related proteins in urine in different stages of DN. The mRNAs of various podocyte associated proteins were found to be significantly higher in urine samples of patients with DN than the controls, and confirmed their usefulness of their quantitation as biomarkers of DN.

**do Nascimento [39] et al.** (2013) estimated urinary mRNA levels of podocyte proteins viz. nephrin, podocalyxin, podocin and synaptopodin. The stages of DN were found to be associated with excretion rate of nephrin, and nephrinuria also predicts pathological albuminuria. No significant difference was found in urinary mRNA levels of podocyte markers in pre-DM subjects than controls.

**Rodrigues [149] et al.** (2014) evaluated the podocyte-associated mRNA profiles in renal tissue and urine of patients with proliferative (PGs) or non-proliferative (NPGs) glomerulopathies. Different profiles of mRNA expression were seen, pointing to a higher degree of intra-renal podocytopenia in the NPGs and of podocyturia in the PGs. The immunosuppressive therapy effectively reduced the urinary levels of podocyte-associated mRNAs.

**dos Santos [150] et al.** (2015) hypothesized that the severity of the histological lesions of lupus nephritis affects podocyte-associated mRNAs profiles in kidney tissue and in urine. Inhibition of podocyte-associated mRNAs in kidney tissue suggests that podocyte injury occurs regardless of severity of lupus nephritis. Increased urinary excretion of podocyte mRNAs, mostly in patients with moderate-to-severe lesions, may reflect a greater burden of glomerular damage with detachment of podocytes into the urine.