1.1 The History of Diabetes Mellitus

The sweet-tasting of urine was first reported in literature around 5 AD as “Madhumeh” in a Sanskrit text credited by two Indian physicians, Susruta and Charuka [Dwivedi and Dwivedi 2007]. Thomas Willis (an English physician, anatomist and physiologist) reported diabetes in medical literature at 1650 BC. The word ‘diabetes’ was first seen in the work of Areteus the Cappadocian (Around 150 AD), he describe that “The disease appears to have got the name diabetes” which is inspired from a Greek word which means a siphon, because the fluid does not remain in the body. William Cullen who was a Scottish physician added the word ‘mellitus’ (A Greek word meaning honey-like) in 1750 BC.

1.2 Definition, Classification, Diagnosis and Etiology

Diabetes is one of the most common non-communicable diseases and one of the most challenging health problems in the twenty-first century [Zimmet et al 2001]. The World Health Organization (WHO) has accredit developmental strategic for interventions. Socio-economic change in society e.g. modernization, including urbanization, westernization of lifestyles, and economic development are transforming the cultural processes uttering to diabetes epidemic. Demographic trends may play a diversifying role (e.g., the increased number of elderly persons), Diabetes is characterized by frequent urination (polyuria), hunger (polyphagia), weight loss, blurred vision, and skin itchiness. Diabetes is associated with long term damage and dysfunction of the beta cells of pancreas, eyes (retinopathy and diabetic cataracts), kidneys (nephropathy), nerves (neuropathy), heart, and blood vessels [American Diabetes Association 1997, 2003]. Attention must paid to diabetes because its associated late complications which lead to macrovascular as well as microvascular complications, drain out the quality of life, along with enormous impact on the economy and productivity of developed and developing nations.

The etiological classification of diabetes has three major subdivisions. Type 1 or juvenile diabetes is an autoimmune disease that destroys the beta cells of the pancreas and
accounts for approximately 5% of diabetes worldwide. Type 2 diabetes or adult-onset diabetes is characterized by hyperinsulinemia in response to insulin resistance of the target tissue. It accounts for approximately 90-95% of diabetes worldwide and it occurs disproportionately among populations adopting a Westernized lifestyle [Baschetti 1998, Gohdes 1996, Mather et al 1985]. Obesity is the most significant risk factor for type 2 diabetes. Approximately 5-10% of diabetes is due to other causes that are often transient rather than chronic. These include gestational diabetes (2-5%), drug or chemically induced diabetes, genetic syndromes, infections, and other endocrine diseases. The current classification of diabetes lists more than 50 specific causes [American Diabetes Association 2010].

1.3 Type 1 Diabetes: Immune-mediated Diabetes
This form of diabetes accounts for only 5-10% of diabetic individuals previously known as the terms insulin dependent diabetes, type 1 diabetes, or juvenile-onset diabetes, results from acellular-mediated autoimmune destruction of the β-cells of the pancreas. Markers of the immune destruction of the β-cells include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD65), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2β. One and more of these autoantibodies are present in 85-90% of immune mediated diabetic individuals. In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (many adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this later stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide. Immune mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age. Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is
not incompatible with the diagnosis. These patients are also prone to other autoimmune disorders such as Graves’ disease, Hashimoto’s, thyroiditis, Addison’s disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia according to the expert committee on the diagnosis and classification of Diabetes mellitus [American Diabetes Association 1997, 2010].

1.4 Type 2 Diabetes
Ranging from predominantly ‘insulin resistance with relative insulin deficiency’ to predominantly ‘an insulin secretary defect with insulin resistance’

This form of diabetes, which accounts for 90-95% of those with diabetes, previously referred to as non-insulin dependent diabetes, type 2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency at least initially, and often throughout their lifetime, these individuals do not need insulin treatment to maintain the blood sugar level. There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur, and patients do not have any of the other causes of diabetes listed above or below. These forms of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages are often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications. Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β-cell function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and / or pharmacological treatment of hyperglycemia but is seldom restored to normal. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. The expert committee on the diagnosis and classification of Diabetes mellitus, American Diabetes Association reported that the this form of diabetes often associated with a strong genetic susceptibility, in comparison of
autoimmune form of type 1 diabetes. However, the genetics of this form of diabetes are complex and not clearly defined [American Diabetes Association 1997, 2010].

1.5 Idiopathic Diabetes

Some forms of type 1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of patients with type 1 diabetes fall into this category, of those who do, most are of African or Asian ancestry. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of strongly inherited, lacks immunological evidence for β-cell autoimmunity, and is not HLA associated. An absolute requirement for insulin replacement therapy in affected patients may come and go [American Diabetes Association 2010].

1.6 Criteria for the Diagnosis of Diabetes mellitus

1. Symptoms of diabetes plus casual plasma glucose concentration ≥200 mg/dl (11.1 mmol/l). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

   OR

2. FPG ≥ 126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 hour.

   OR

3. 2-hour postload glucose ≥ 200 mg/dl (11.1 mmmol/l) during an OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

   OR

4. HbA1C ≥ 6.5%. The test should be performed in a laboratory using recommended methods by suggested by the expert committee of American Diabetes Association on diagnosis and classification of Diabetes mellitus [American Diabetes Association 2010, 2011].
1.7 Diabetic Nephropathy

Diabetic nephropathy in type 2 diabetic patients is a disease of heterogeneous origin, the clinical manifestations of diabetic nephropathy are strongly related with the structural alterations, especially with the degree of mesangial expansion in both type 1 and type 2 diabetic patients.

In type 2 diabetic patients the characteristics are more complex then type 1 patients glomerulopathies including minimal lesion nephropathy, chronic glomerulonephritis, and mesangial proliferative glomerulonephritis alone or superimposed to other structural abnormalities. Recently changes in the structure and number of podocytes have been reported from numerous studies.

1.8 Classification of Diabetic Nephropathy

Diabetic nephropathy can be classified in to the following categories on the basis of histological lesions.

1.8.1 Glomerular Lesions

1.8.1.1 Class I: This class includes glomerular basement membrane thickening, Electron microscopic observation suggest that at initial stage glumerulous basement membrane thickening must be more than 430 nm and 395 nm, for older and 520 nm, 471 nm for adult male and female respectively. Isolated glomerular basement membrane shows extent of thickening is only mild and do not meet criteria of classes II through IV. GBM thickening is a consequence of extracellular matrix accumulation, with increased deposition of normal extracellular matrix. GBM thickening is a characteristic of incipient nephropathy in type 1 and type 2 diabetic patients.

1.8.1.2 Class II, Mesangial Expansion, Mild (IIa) or Severe (IIb): Also known as “diffuse diabetic glomerulosclerosis.” The difference between mild and severe mesangial expansion is based on whether the expanded mesangial area is smaller or larger than the mean area of a capillary lumen furthermore if mesangial lesion are in less than 25% area it is classified in IIa and if mesangial expansion is more than 25% area, it is classified in class IIb.
1.8.1.3 Class III Nodular Sclerosis (Kimmelstiel–Wilson Lesions): If the biopsy is found one Kimmelstiel–Wilson lesion and does not have more than 50% global glomerulosclerosis, it is classified as class III. These lesions includes focal, lobular, round to oval mesangial lesions with an acellular, hyaline/matrix core, rounded peripherally by sparse, crescent-shaped mesangial nuclei. Moreover class III typically characterized by accumulation of mesangial matrix with collagen fibrils, small lipid particles, and cellular debris with decreased mesangial cells at central are of glomerular tuft in both type 1 and type 2 diabetic patients.

1.8.1.4 Class IV, Advanced Diabetic Glomerulosclerosis: In this class glomerulosclerosis is seen in more than 50%, with other clinical or pathologic evidence leading to sclerosis. This stage is further characterized by huge accumulation of extracellular matrix proteins such as collagen types I, III, and IV and fibronectin in the mesangial space. Class IV is significantly correlated with protenuria.

1.8.2 Tubular and other lesions

1.8.2.1 Tubular Lesions: Tubular lesions are characterized by the tubular basement membrane thickening of nonatrophiic tubules which is manifest from the development of class II glomerular lesions and becomes more striking in class III and IV. Interstitial fibrosis and tubular atrophy are serious lesions with glomerular lesions which lead to faster progression of nephropathy and early incidences of kidney failure.

1.8.2.2 Vascular Lesions: Vascular lesions are characterized by either hyalinosis of efferent (more common) and afferent arterioles (less common), or both. Efferent arterioles hyalinosis is more frequently seen in diabetic patients distinguish from hyalinosis by systemic hypertension in non diabetic subjects.

1.8.2.3 Other Glomerular Lesions: In this class non sense lesion occurs and it difficult to classify ideotype lesion, biopsies may found intramural accumulations of affectionated plasma proteins and lipids within renal arterioles, glomerular capillaries, Bowman’s capsule, or proximal convoluted tubules. Moreover diabetic nephropathy does not exhibits all classes lesion and scares are heterogeneous in nature certain time rare type lesions are also found like glomerulotubular junctions with focal adhesions called “tip lesions” and atrophic tubules with no observable glomerular opening (so-called “atubular
glomeruli”). These lesions are more frequently found in advanced stages of nephropathy and overt proteinuria [Tervaert et al 2010].

1.9 Criteria for the Diagnosis of Diabetic Nephropathy
Numerous methodology has been suggested to diagnose and prognose diabetic nephropathy, which includes kidney volume by ultrasound, identification of renal scars through renal biopsies, glomerular filtration rate estimation and proteinuria. GFR and proteinuria are clinically adopted and trustworthy methods used in routine clinical practice.

1.9.1 GFR Criterion
Chronic kidney failure (CKF) refers to a progressive and irreversible loss of renal function. This usually occurs when the glomerular filtration rate (GFR) is reduced to at least 50-60 ml/min/1.73m², has been classified into five stages; shown in Figure 1.1.

Stage 1: Patients with normal glomerular filtration rate (GFR) but with some evidence of kidney damage such as microalbuminuria/proteinuria, haematuria or histological changes.
Stage 2: Mild diabetic nephropathy with a GFR ranging from 89 to 60 ml/min/1.73m²
Stage 3: Moderate diabetic nephropathy with a GFR ranging from 59 to 30 ml/min/1.73m²
Stage 4: Severe diabetic nephropathy with a GFR ranging from 29 to 15 ml/min/1.73m²
Stage 5: Kidney failure when GFR is < 15 ml/min/1.73m²
This stage is when renal replacement therapy (RRT) in the form of dialysis or transplantation has to be considered.

Figure 1.1 Time scale for stages and progression of diabetic nephropathy.
1.9.2 Spot Urine Microalbumin Criterion
A test for the presence of microalbumin is suggested by several nephrology societies and committee as shown in Table 1.1, diagnosing type 2 diabetic patients widely accepted screening for microalbuminuria can be performed by three methods:

1. Measurement of the albumin-to-creatinine ratio in a random spot collection
2. 24-hour collection with creatinine, allowing the simultaneous measurement of creatinine clearance
3. Timed (e.g., 4-hour or overnight) collection

The albumin-to-creatinine ratio is often found to be the reliable method and possesses least contamination, provides accurate information [American Diabetes Association 2010, 2011].

Table 1.1 Cutoff criterions for albumin excretion

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Category</th>
<th>Spot collection (µg/mg creatinine)</th>
<th>24-h collection (mg/24 h)</th>
<th>Timed collection (µg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal</td>
<td>&lt;30</td>
<td>&lt;30</td>
<td>&lt;20</td>
</tr>
<tr>
<td>3.</td>
<td>Clinical albuminuria</td>
<td>≥300</td>
<td>≥300</td>
<td>≥200</td>
</tr>
</tbody>
</table>

1.10 Worldwide Prevalence of Diabetes
The prevalence of diabetes has already achieved epidemic proportion with more than 285 million patients worldwide compared to 171 million in 2000 as shown in Figure 1.3. India has the largest diabetic prevalence, the growth rate in percentage is 71.3%, followed by China (44.9%), USA (34.3%) and Russia (7.3%) as shown in Figure 1.2. There are 6.6% people with diabetes mellitus worldwide; 143 million of them are women and 142 million are men consequently, the worldwide prevalence of diabetes among adults (20–79 years of age) is expected to increase up to about 439 million by the year 2030. Furthermore according to the projection estimate from 2010 to 2030, shown in Figure 1.3 and 1.4.
Figure 1.2 Demographic presentation of diabetic prevalence

Figure 1.3 Diabetic prevalence and projection from 2010 to 2030
There will be an increase of 69% in the number of adult diabetic patients for the developing countries, while for developed countries it is expected to increase by 20%. Furthermore, prognosis for the year 2030 is demonstrating that countries with high population numbers, such as China, India and USA, will have a bigger increase in both absolute number and percentage of diabetic cases in population [George and Cebioglu 2010, Shaw et al 2010]. In addition recently it has been reported diabetic patients without complications and with complications spends 11 to 75% and 6% to 300% of per-capita income respectively in country like India and China [Goldhaber-Fiebert et al 2010], which increases extra health economic burden on country.

### 1.11 Prevalence of Diabetes in India

Recently in 2010, India has the highest absolute number of registered diabetic patients, as shown in Figure 1.2. Since last few decades India is continuously facing a grave healthy care burden due to the high prevalence of type 2 diabetes and its squeal [Ramachandran et al 2002]. Studies conducted in India in the last decade have suggested that not only the prevalence of type 2 diabetes is high, but also it is increasing with associated complications rapidly, type 2 diabetes is 4-6 time higher in urban area than rural areas.

![Figure 1.4 Diabetic prevalence in male and female](image)
[Mehta et al 2009]. In India the crude prevalence in type 2 diabetic and pre-diabetic among adults was reported 12.1% in 2009. Type 2 diabetes generally rose with age in both genders, reaching 43.3% among the 36 to 50 year age-group and 50.0% among the ≥50 year age-group [Balagopal et al 2008]. According to estimation in 2030 there will be 87 millions young adults people of aged 20–79 years with diabetes mellitus [Shaw et al 2010].

1.12 Diabetic and Non-diabetic Nephropathy: An Overview
Diabetic nephropathy is leading cause of death and accounts largest proportion among secondary complications as shown in Figure 1.5. Diabetic nephropathy is also became serious threat and medical burden to the epidemic proportion with the beginning of this millennium. International Society of Nephrology with the association of International Federation of Kidney Foundations has specially focused on diabetic nephropathy with the slogan “Protect your kidneys, Control diabetes” on 11 March 2010 at World Kidney Day celebration [Tang 2010].

![Figure 1.5 Secondary co-complications of type 2 diabetes](image-url)
In estimation USA will have increased medical burden of 300,000 patients with nephropathy by 2030 which was 40,000 in 2000 [Hovind 2005]. Recently overall prevalence of diabetic nephropathy is 25% in USA and 32.5% in India [George 2010]. Moreover, prevalence of type 2 diabetic nephropathy in Indian population has been reported 26.9% for microalbuminuric patients and 2.2% for overt nephropathy [Unnikrishnan et al 2007]. In India incidences of end stage renal disease was reported crude 151 and age adjusted 232 per million population and around 44% who face renal failure are from diabetic background [Modi et al 2006, Dabla 2010].

Furthermore chronic renal failure patients do not develop uremic symptoms until the GFR declines up to 25-20 ml/min, therefore prevalence of chronic renal failure is difficult. Screening of individuals for diabetic nephropathy would have to rely on clinical examination, biochemical investigations and/or urinalysis of at risk individuals. A study by National Kidney Foundation (USA) namely KEEP (Kidney Early Evaluation Program) has stated that those patients who are at the risk of developing nephropathy, ~71.4% have been found to having at least one abnormality [O’Connor et al 2005, Brown et al 2003]. These data suggest there is immediate need to pay attention for diabetes and its secondary complications with special reference to diabetic nephropathy.