Introduction of Diabetes

Diabetes mellitus encompasses a heterogeneous group of metabolic disorders that are commonly characterized by hyperglycemia in the blood. Under healthy physiological conditions, insulin secreted from the pancreas remove excess glucose from the blood, thereby maintaining the blood sugar level to normal values. During diabetic condition, the presence of elevated blood sugar is because of either insulin not being produced/insufficient produced or it is not as efficient as it should be (Gavin et al., 2002). Diabetes mellitus impairs the ability to metabolize fat, carbohydrates, and proteins leading to morbidity. Hyperglycemia in the blood frequently causes various secondary complications eventually resulting in mortality.

Prevalence of Diabetes

Diabetes is a highly epidemic and one of the biggest health threats worldwide significantly affecting an individual’s life. According to IDF (International Diabetes Federation) Diabetes Atlas report of 2015, 415 million people aged between 20-79 years are suffering from diabetes in the world. By 2040, it is expected to rise nearly by 55% indicating 642 million individuals with diabetes (Figure 1.1). Due to such high prevalence, diabetes is the huge health burden that makes to spend 12% of the global health expenditure on it. The surveys reported 5 million deaths globally in 2015 because of diabetes. In the present scenario, India is the second highest country having 69.2 million people with diabetes which is thought to reach at 123.5 million by 2040, i.e., 78.5% increase (IDF, 2015). The incidences of diabetes greatly vary among different Indian regions showing prevalence from as low as 0.4% to as high as 19.8% (Figure 1.2) (Unnikrishnan, Anjana, and Mohan, 2016). Besides, it is a fact that there is a vast population of as much as 50% is undiagnosed which is a quite serious problem (IDF, 2015).
Figure 1.1: The prevalence of diabetes worldwide and per region showing estimated diabetic individuals in 2015 and 2040 (IDF, 2015)
Introduction

Figure 1.2: The prevalence of diabetes mellitus in rural (left) and urban (right) populations of India in 2014 (Unnikrishnan, Anjana, and Mohan, 2016).

Classification of Diabetes mellitus

The two major forms of diabetes mellitus are type 1 diabetes (T1D) and type 2 diabetes (T2D).

1. T1D accounts for 5-10% of the total diabetes cases which is primarily an autoimmune disorder. It is caused by the autoimmunological destruction of the insulin-producing β cells of the pancreas, and genetic predisposition plays a chief role in the disease etiology having HLA as the major susceptibility gene (Concannon et al., 1998). Among T1D individuals, varying degree of the β cell deaths leads to insufficient insulin production or absolute insulin deficiency. So, most of the patients rely on insulin supplements due to which it is also known as IDDM (Insulin Dependent Diabetes Mellitus).

2. T2D, the most common form of diabetes, accounts for approximately 90-95% of all diabetic patients and affects 10–20% of people in many countries that are aged over 45 years (Horikawa et al., 2000). T2D or adult-onset diabetes indicates individuals with the inability of insulin to be effective on its target peripheral tissues as it should be known as insulin resistance. So, during the early period or
sometimes till the lifetime the patients do not need insulin treatment, and hence it is also called a NIDDM (Non-Insulin Dependent Diabetes Mellitus) (ADA, 2014). T2D is a highly multifaceted disease primarily associated with obesity and sedentary lifestyle along with polygenic complex genetic predisposition. As our work was concentrated on the T2D, we would be talking mostly about T2D.

Apart from these two forms of diabetes, the other types of diabetes include Maturity Onset Diabetes for Youth (MODY), neonatal diabetes, gestational diabetes (GDM), drug or chemical induced diabetes and other autoimmune as well as genetic syndrome induced diabetes (ADA, 2014).

**Symptoms of Diabetes**

Prediabetes is the condition encountered before diabetes where the person is not aware of his impaired glucose tolerance (IGT) or impaired fasting glucose (IFG). At this stage, the small increase in blood sugar level is usually asymptomatic making it undiagnosed most of the times. Further, if situation left untreated, it would develop diabetes where marked hyperglycemia will show common symptoms like polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Often these symptoms are accompanied by fatigue, tingling in the extremities, weight loss, and blurred vision (IDF, 2015). Since these signs remain less evident in many individuals with T2D, they stay unaware of their condition for a long time and may recognize it when the situation becomes severe.

**Etiology of Diabetes**

The risk of developing T2D rises evidently with age (> 45 years), obesity (BMI > 30), and lack of physical activity (ADA, 2004). Moreover, individuals with a family history of T2D and people from specific races/ethnicities (such as Hispanic, Native American and Australian Indigenous populations, and African American, and Asian populations) are more prone to the disease. These subgroups have particular genetic attributes that either directly causes diabetes or are associated with it (Chen et al., 2012). In females, previous gestational diabetes or polycystic ovary syndrome elevates the occurrence of T2D further. The other factors that may play a role in disease susceptibility are hypertension, dyslipidemia, IGT or IFG (IDF, 2015; ADA, 2004).
T2D is a multifactorial and highly complex disorder due to which its etiology is not entirely revealed. However, as mentioned certain factors apparently increase the risk of developing T2D (Figure 1.3). Moreover, the presence of a risk factor(s) does not assure the development of diabetes. It seems like more the risk factors an individual possesses; there are greater chances of developing or having diabetes. On the contrary, an asymptomatic individual without having any risk factors may develop diabetes, but the chances are relatively low (ADA, 2004).

**Diagnosis of Diabetes**

Since long, the classic tests for diagnosing diabetes were based on glucose criteria, either the Fasting Plasma Glucose (FPG) or the 75-g Oral Glucose Tolerance Test (OGTT) and are yet in standard practice. Later, The Expert Committee after an extensive review recommended the use of the HbA1C (glycated hemoglobin) test for diagnosing diabetes (ADA, 2014). Usually, the FPG estimation is done in the morning with an empty stomach because it requires the fasting of minimum 8 hrs. The OGTT involves quantification of the blood glucose before and 2 hrs after the administration of the equivalent grams of glucose to the body weight of an individual. Thus, it provides the information about the processing of glucose. Moreover, Random Plasma Glucose (RPG) Test is also used to diagnose diabetes that identifies the glucose levels at any time of the day. The test is usually done when diabetes symptoms are observed. The A1C levels represent the average blood glucose for the past 2 to 3 months. The
test provides the information regarding sugar levels for the previous three months irrespective of the existing sugar level due to which nowadays it is highly recommended by the physicians.

The Expert Committee on Diagnosis and Classification of Diabetes Mellitus identified an intermediate group of those who have glucose levels higher than the considered normal yet do not meet the criteria for diabetes (ADA, 2014). They have been regarded as prediabetic individuals, possessing the high risk for the development of diabetes. The diagnostic criteria used for diabetes are illustrated in Figure 1.4 for all the three tests as recommended by ADA.

![Figure 1.4: Diagnostic criteria of diabetes and recommended threshold values of three classic diagnostic tests for Normal, Prediabetes and Diabetes individuals (ADA guideline)](image)

**Pathophysiology of Type 2 Diabetes**

The current understanding says that consequence of the actions and interactions of various non-genetic (e.g., obesity, sedentary lifestyle, age, hypertension) along with genetic susceptibility factors cause diabetes especially T2D (Frazer *et al*., 2009; Doria, 2010). The underlining precise molecular mechanism of T2D pathology is yet to reveal due to highly multifaceted nature of the disease. However, it is evidently shown that insulin resistance, oxidative stress, pancreatic β-cell dysfunction and decreased β-cell mass are the key mechanisms involved in the T2D pathogenesis.
Insulin Secretion: Insulin secretion from the β-cells is stimulated by increased glucose levels in the blood which is regulated by ATP coupled potassium channel. As illustrated in Figure 1.5 (Left), at low plasma glucose level, the decreased ratio of ATP/Mg-ADP will enhance KATP channel opening. Subsequently, the cell membrane becomes hyperpolarized that prevents opening of voltage-gated calcium channel, Ca2+ influx, and finally insulin secretion. Conversely, during increased plasma glucose, it gets transported into the β cell via GLUT2 followed by the elevated glucose metabolism (Figure 1.5 (Right)). Consequently, ATP/Mg-ADP ratio increases, resulting in KATP channel closure, membrane depolarization, the opening of calcium channels leading to the Ca2+ influx, and in turn insulin secretion (Ashcroft et al., 1994; Lang and Light, 2010).

Insulin Signalling and Insulin Resistance: Insulin induces glucose uptake in muscle and adipose tissues via GLUT4, whereas, it prevents gluconeogenesis and glycogenolysis and induces glycogen synthesis in the liver to maintain fasting glucose levels. The insulin first binds to its receptor called IR (Insulin receptor) that activates a cascade on intracellular events to maintain glucose homeostasis. The activated IR phosphorylates insulin receptor substrate (IRS) proteins, which are responsible for stimulating the phosphatidylinositol 3-kinase (PI3K)-AKT/protein kinase B (PKB) pathway (Figure 1.6). Finally, this pathway through cascade mechanism performs the majority of metabolic actions of insulin, like inducing glucose uptake via GLUT4.
fatty acid synthesis, glycogen synthesis, and preventing gluconeogenesis and glycogenolysis (Saltiel and Kahn, 2001).

(a)

Figure 1.6: Mechanisms of insulin signaling and lipid-induced insulin resistance in (a) muscle tissues and (b) liver. (DAG, Diacylglycerol; IRS, Insulin Receptor Substrate; GS, Glycogen Synthase; GSK3, Glycogen Synthase Kinase 3 beta; PI3K, phosphatidylinositol 3-kinase) (Bays et al., 2013).
Insulin resistance can occur due to impairment in insulin signaling at many levels like lower receptor concentration and kinase activity, phosphorylation of IRS-1 and IRS-2, PI(3)K activity, GLUT4 translocation, and the activity of intracellular enzymes (Saltiel and Kahn, 2001). High lipid level increases the fatty-acid oxidation in the muscle tissue as well as the liver that accumulates high levels of diacylglycerol (DAG), ceramides and acetyl Co-A in the cell. DAG in the muscle activates protein kinase C θ (PKC θ), results in serine phosphorylation of IRS-1/2, and impairs translocation of GLUT4 to the membrane (Figure 1.6 (a)). Whereas, in the liver, DAG stimulates PKC ε that cause malfunctioning of insulin signaling and diminished regulation of hepatic glucose production (Bays et al., 2013). Thus, reduction in glucose uptake and its altered metabolism cause hyperglycemia in the blood.

**Diabetic Complications**

Long-standing hyperglycemia is an important responsible factor for the development of several diabetic secondary complications such as ketoacidosis, cardiovascular diseases, retinopathy, neuropathy, and nephropathy (Gariano and Gardner, 2005). These complications are the primary cause of the morbidity and mortality due to diabetes. The diabetic people are at risk of other complications like genitourinary and sexual dysfunction, hypertension, and deform lipoprotein metabolism.

The secondary complications of diabetes are chiefly classified as microvascular and macrovascular complications. The microvascular complications of diabetes include retinopathy that may potentially lead to loss of vision; nephropathy leading to renal failure; neuropathy including the risk of amputations, foot ulcers, and Charcot's joints. On the other hand, macrovascular complications include various cardiovascular diseases like ischemic heart disease, peripheral vascular disease, and cerebrovascular disease. The chronic hyperglycemia triggers chiefly five intracellular mechanisms that appear to be responsible for causing secondary microvascular and macrovascular complications. Specific cell types like endothelial cells in the retinal capillaries, mesangial cells in the renal glomerulus, and neurons and Schwann cells in peripheral nerves encounter hyperglycemia in the cell during diabetic condition since they are not able to decrease the transport of glucose in the cell. This intracellular hyperglycaemia (i) increases polyol pathway flux (ii) increases formation of advanced glycation end products (AGEs) (iii) induces the activation of protein kinase C (PKC) (iv) increases hexosamine
pathway flux and subsequent alterations of proteins caused by overproduced N-acetylglucosamine and (v) increases oxidative stress (Brownlee, 2005).

**Diabetic Retinopathy**

Diabetic retinopathy (DR) is a serious eye problem in the world, and also the most common secondary complication of diabetes. It is one of the leading causes of blindness in working-aged adults around the globe (Yau et al., 2012). It damages the retina, a light-sensitive and a vital tissue in vision located at the back of the eye, mainly by microaneurysms, hyperpermeable vasculature, and neovascularization in the retina. As shown in Figure 1.7, the surveys estimate 145 million people suffering from DR worldwide that is predicted to reach 224 million by 2040, i.e., 35% of the diabetic population has some form of DR (IDF, 2015). However, the DR prevalence varies around the globe where it is found comparatively less among Asians than to Caucasians. In India, the scenario is better affecting 13-18% of urban and 9–10% of the rural diabetic population (Raman et al., 2016). More than 60% of the T2D patients develop DR after 20 years of diabetes irrespective of their diabetic control which was confirmed by other studies including in India (Aiello et al., 1998; Bansal, Gupta, & Kotecha, 2013).

![Figure 1.7: The estimated global prevalence of DR in 2015 and 2040 (IDF, 2015)](image)
DR risk factors

DR etiology involves the interplay between various conventional risk factors like long diabetes duration, poorly controlled hyperglycemia, hypertension, and hyperlipidemia that are mainly related to diabetic condition (Kuo et al., 2014). Nonetheless, it is highly evident that the risk of developing DR is not completely based upon these procedural and clinical features. In the current scenario, the genetic factors are well established in explaining part of the excessive risk of DR development independently of traditional clinical factors (Tang et al., 2013). However, being highly multifaceted disease, the entire molecular mechanism of the DR pathogenesis is yet unrevealed.

Fundamentals of DR

The retina is positioned at the back of the eye that is made up of a light-sensitive thin layer. It mainly consists of retinal pigment epithelial cells, red and cone cells, and neurons. The light rays are focused onto the retina, where rode and cone cells process them and signals generated are transmitted to the brain that ultimately interprets it as an image. The macula, as shown in normal retina (Figure 1.8), is a small area at the center of the retina which plays a crucial role in the vision process. Beside the macula dense disk-like structure from where all neurons of retina pass towards brain which is known as an optical disc (Figure 1.8). The surrounding area of it identified as peripheral retina that aids the peripheral vision.

Figure 1.8: The illustration of Normal retina and stages of Diabetic retinopathy.
As previously mentioned DR involves damage to the tiny blood vessels in the retina, leakage, and bleeding of these tiny vessels, finally triggering the formation of the new abnormal vasculature. Hence, DR arises when retinal capillaries swell, leak fluid or blood, or close off that is responsible for microaneurisms. In an advanced stage, the hyperpermeable new blood vessels are being developed on the retinal surface. Some of the major symptoms include blurred vision, blank or dark areas in the vision field, spots, dots or dark strings floating, poor night vision; and vision loss.

**Clinical Classification of DR**

DR initiates from a mild non-proliferative condition that characterized by swelling higher vascular permeability of the retinal vessels and progresses to moderate and severe nonproliferative diabetic retinopathy (NPDR), characterized by ischemia-induce microaneurisms. Later, the situation leads to the neovascularization to circumvent the hypoxia which is also hyperpermeable causing hemorrhages and leakage of plasma proteins/lipids, known as Proliferative diabetic retinopathy (PDR). The edema on macula can occur at any of the stages of DR that is the chief cause of central vision loss (Fong *et al*., 2004; Ozturk *et al*., 2009). Besides, if PDR remained untreated, it may lead to vision loss.

NPDR is the primary stage that involves microaneurysms, hard exudates and/or hemorrhages, whereas, PDR chiefly identified by the impaired new vasculature formation in the area of the retina. The PDR pathology precisely includes abnormal angiogenesis and increasing the extracellular matrix (ECM) leading to the formation of fibrovascular membranes (FVM) at the vitreoretinal interface responsible for basement membrane thickening. The contraction in fibrovascular tissue results ultimately in severe and often irreversible vision loss through vitreous hemorrhage and traction retinal detachment (Ozturk *et al*., 2009; El- Asrar *et al*., 2013). Central vision is mainly impaired by DME (diabetic macular edema).

**Diagnosis of the DR and its severity grading**

DR and DME are clinically detected by performing a comprehensive eye examination that includes visual acuity test, dilated eye examination, tonometry, and fluorescein angiography. Table 1.1 illustrates the various diagnosing criteria for identifying the severity of DR (Wilkinson *et al*., 2003).
1. **Visual acuity test.** This traditional eye chart is shown to the patient, and he has to read. The test enables to determine the degree of damage to the central vision.

2. **Dilated eye exam.** An ophthalmologist can see more of the inside of eyes through the dilated pupils that allow identification of the disease signs. A special magnifying lens or ophthalmoscope is used to examine the retina and optic nerve for any signs of damage.

3. **Tonometry.** It measures the pressure within the eye for which anesthetizing drops may be applied to eyes.

4. **Fluorescein angiography.** In this test, a particular dye is injected into the blood and pictures of the eye are captured as the dye passes through the blood capillaries in the retina. The test allows the sensitive and specific detection and evaluation of any abnormalities related to retina including DR.

### Table 1.1: Diagnosis criteria of the DR disease severity based on ETDRS classification

<table>
<thead>
<tr>
<th>Proposed Disease Severity Level</th>
<th>Findings Observable Upon Retina Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>No apparent retinopathy</td>
<td>No abnormalities</td>
</tr>
<tr>
<td>Mild Non-Proliferative Diabetic Retinopathy (Mild NPDR)</td>
<td>Microaneurysms only</td>
</tr>
<tr>
<td>Moderate Non-Proliferative Diabetic Retinopathy (Mod NPDR)</td>
<td>More than just microaneurysms but less than NPDR</td>
</tr>
</tbody>
</table>

Any of the following:
- More than 20 intraretinal hemorrhages in each of 4 quadrants
- Definite venous beading in 2+ quadrants
- Prominent IRMA in 1+ quadrant

Moreover, no signs of proliferative retinopathy

<table>
<thead>
<tr>
<th>Severe Non-Proliferative Diabetic Retinopathy (SNPDR)</th>
<th>One or more of the following:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neovascularization</td>
</tr>
<tr>
<td></td>
<td>Vitreous/preretinal hemorrhage</td>
</tr>
</tbody>
</table>

| Proliferative Diabetic Retinopathy (PDR) | |
|-----------------------------------------| |

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Current Treatments for DR

DR treatment is prescribed as per the stage or severity of the disease. Usually, during the initial three stages of DR, there is no treatment required, unless DME is observed. Till then the patient is asked to maintain the glycemic control and blood pressure.

**Anti-VEGF Therapy:** Since VEGF plays a crucial role in the DR pathogenesis, Anti-VEGF therapies are successful. It includes antibodies such as bevacizumab, and ranibizumab, or orally-available small molecules that inhibit the tyrosine kinases activated by VEGF (Marozas and Fort, 2014). The efficacy of treatment with ranibizumab and bevacizumab confirmed the VEGF contribution to the DME and DR.

**Laser Surgery:** As disease exaggerated, leakage of fluid and blood from retinal capillaries can lead to DME, which is treated with laser surgery. This procedure is called photocoagulation or focal laser treatment (Marozas and Fort, 2014). Enormously, small laser burns are created to seal the leaks and reduce the amount of fluid in the retina.

**Vitrectomy:** This treatment is needed in an advanced stage such as severe bleeding, and retinal detachment to restore the vision by removing a significant amount of blood from the center of the eye (vitreous gel) (Smiddy and Flynn, 1999). A vitrectomy is executed under local or general anesthesia that involves a tiny incision in the eye of a patient. Subsequently, the vitreous gel clouded with blood is removed using a specialized instrument which is replaced by a salt solution. Post surgery, wearing an eye patch and medicated drops are required to protect against infection.