Chapter-1  INTRODUCTION

Diabetes mellitus is a metabolic disorder, which makes it difficult for the cells of the body to use glucose for energy. It results from reduced ability, expression and action of insulin to stimulate glucose transport. If untreated diabetic condition continues for long it results in chronic hyperglycemia which is closely associated with long term complications, which include damage, dysfunction and failure of various organs including eyes, kidneys, nerves, heart and blood vessels. The majority of cases of diabetes fall into two broad etiopathogenic categories i.e. type 1 and type 2 diabetes. In type 1 diabetes, hyperglycemia results from inadequate secretion of insulin, by islets of Langerhans in the pancreas. The more prevalent type 2 diabetes results from combination of insulin insensitivity and inadequate compensatory insulin secretory response. In type 2 diabetes, the degree of hyperglycemia is sufficient enough to cause pathologic and functional changes in various target tissues. These pathological changes may be present in the subject for a long time without any clinical symptoms prior to detection of diabetes.

Diabetes is rapidly becoming the epidemic of 21st century. It accounts for 3.8 million deaths per year globally. According to the latest figures from International Diabetes Federation’s Diabetes Atlas, a staggering 246 million people across the world are affected by diabetes. Although diabetes is prevalent throughout the world, the prevalence rate of diabetes in India is significantly higher in terms of the number of people affected. Infact, India leads the global top ten countries in the world in terms of highest number of people with diabetes with a current figure of 40.9 million. For this reason India is now referred as diabetic capital of the world. The staggering growth rate of diabetic patients and its far-reaching societal and economic consequences is a public health issue of clinical importance.

A number of factors and their complex interplay predispose the individual towards the risk of diabetes. Genetics, among these factors plays a cardinal role in development of disease pathology. An extensive search has been carried out to
identify and decipher clues in our genetic makeup and develop “genetic landscape of diabetes” that may explain why some people are at a higher risk for diabetes than others. Family history, which captures these genomic interactions, is considered clinically useful to identify people at high risk. A family history of diabetes is independently and significantly associated with the development of diabetes itself, even after adjusting for other risk factors. In general, a family history of diabetes in a first-degree relative doubles a person’s risk of developing diabetes. This is attested by a number of studies carried out in this field.

Oxidative stress represents an important pathway for the destruction of cells and production of Reactive Oxygen Species (ROS) that can result in cellular injury through cell membrane lipid destruction and cleavage of DNA. This can lead to various derangements in the body including diabetes. A number of recent studies have shown the presence of oxidative stress in first-degree relatives of type 1 diabetic patients exhibiting the contributory effect of oxidative stress towards development of diabetes. In contrast to this, a limited number of studies have been done to identify relationship between family history and development of type 2 diabetes. Hence, the present thesis is focused on identifying and gaining better understanding of relationship, if any, between family history and type 2 diabetes. The present thesis entails study of various markers of oxidative stress in first degree relatives of type 2 diabetes to understand relationship between oxidative stress, family history and susceptibility towards type 2 diabetes.

The introduction of this thesis is divided into two sections. The first section describes the role of insulin and impaired glucose tolerance, how they are linked to development of diabetes. This section also includes statistical prevalence of diabetes, factors responsible for diabetes, complications associated with diabetes and pathways explaining diabetic complications. The second section is focused on the genetic makeup, role of family history, involvement of oxidative stress in the etiology of the disease and the interrelationship between all of these factors. These two sections complete our introduction providing the full explanation on diabetes, family background and oxidative stress.
1.0 Insulin and its biological role

Insulin is a natural hormone made by the pancreas. It consists of two polypeptide chains, chain A has 21 amino acids and chain B has 30 amino acids (humans), two disulphide bridges tether the chain and chain A contain internal disulphide bridges. The major role of insulin is to counter the concerted action of number of hyperglycemia generating hormones and to maintain blood glucose level towards normalcy. Insulin plays a significant role in promoting short-term metabolic activities and its long-term growth-promoting action by binding to the insulin receptor on cell surfaces. Insulin increases the transport of glucose into fat and muscle cells by promoting movement of the glucose transporter GLUT4 from the interior of the cell to the cell membrane (Saltiel AR et al, 2001)

This hormone, the only one that reduces blood glucose level, is rapidly cleared from the blood circulation and inactivated by specific insulin-metabolizing enzymes in the cells of the liver and other tissues. It also plays important role in keeping the blood glucose level within acceptable limits.

1.1 How does insulin facilitates entry of glucose

Insulin secretion from pancreatic beta cells is regulated by interactions between a variety of nutrients, hormones and neurotransmitters. Glucose is predominant physiologic nutrient secretagogue, and its ability to elicit an insulin secretory response depends on the ability of the beta cell to metabolize the glucose and generate intermediates, including ATP, which results in membrane depolarization via the closing of ATP dependent potassium channels. In the absence of insulin, the glucose transporters are present in cytoplasmic vesicles. Binding of insulin to receptors on such cells leads rapidly to fusion of those vesicles with the plasma membrane and insertion of the glucose transporters, thereby giving the cell an ability to efficiently take up glucose.

Insulin Receptors are transmembrane receptors on the outer part of a cell that are activated by insulin and allow the cell to join or bind with insulin that is in the blood. They are formed by two subunits linked by disulphide bonds. When the cell and insulin bind together, the cell can take glucose from the blood and use it for energy.
Figure 1 Structure Mature human Insulin
The main activity of activation of the insulin receptor is inducing glucose uptake. For this reason "insulin insensitivity", or a decrease in insulin receptor signaling, leads to diabetes mellitus type 2 - the cells are unable to take up glucose, and the result is hyperglycemia (an increase in circulating glucose), and all the squeal which result from diabetes.

1.2 Insulin resistance

Insulin resistance is a state in which a given concentration of insulin produces less than expected biological effect. Insulin resistance or the lack of physiological response of peripheral tissues to the action of insulin plays a major pathogenic role in the development of a number of metabolic and hemodynamic disturbances known as metabolic syndrome (Sivitz WI, 2004; Ascaso JF et al, 2003). This metabolic syndrome may include any or all of the following conditions

- dyslipidemia
- hypertension
- glucose intolerance or type 2 diabetes
- hyperuricemia
- abdominal obesity
- hypercoagulability with impaired fibrinolysis
- atherosclerosis
- fatty liver
- cardiovascular diseases

Insulin resistance can progress to full type 2 diabetes mellitus. This is often seen when hyperglycemia develops after the meal, when pancreatic cell are unable to produce sufficient insulin for maintenance of normal levels of blood sugar. This inability of beta cells to produce sufficient insulin in a condition of hyperglycemia characterizes the transition from insulin resistance to type 2 diabetes (McGarry J, 2002).
Normal Glucose Tolerance

Impaired Glucose Tolerance

Type 2 Diabetes

β-Cell dysfunctioning

Insulin resistance

Age
Physical activity
Obesity
Heredity

Physical Activity
Weight reduction

Figure 2 Impaired Glucose Tolerance
The cause of insulin resistance is not yet fully understood. It is thought to be due to genetic factors including ethnicity, and partly to lifestyle, such as excessive calorie intake and inadequate exercise. Cross sectional studies carried out on first degree relatives of type 2 diabetics have shown increased insulin resistance, associated cardiovascular risk factors and abnormalities of fibrinolysis and coagulation (Herlihy OM et al., 2002).

1.3 Impaired Glucose Tolerance

Impaired Glucose Tolerance (IGT) or impaired fasting glucose (IFG) refers to a metabolic state intermediate between normal glucose homeostasis and diabetes. It has been defined as blood glucose level greater than or equal to 110 mg/dl but less than 126mg/dl. It may or may not progress to diabetes mellitus and in some cases, blood glucose level may return to normal. This is not a disease type and is only a stage between normal and diabetic.

Extensive researches have been done on diabetes during last 25 years resulting in vast expansion of knowledge. Nowadays diabetes is referred to as group of metabolic disorders which are caused due to decrease in insulin concentration in body and/or defective receptors for insulin action, or both, affecting metabolism of carbohydrates, proteins, fats, water and electrolytes. At the onset of disease these metabolic disorders are of reversible nature and can be delayed by strict sugar control. In the long run, with uncontrolled hyperglycemia, these metabolic disorders become associated with irreversible and permanent anatomical and physiological changes leading to various long term complications involving eyes, kidney, nervous tissues, blood and vascular tissues.

1.4 Classification of Diabetes

In 1997, ADA issued new diagnostic and classification criteria. In 2003, modifications were made regarding the diagnosis of impaired fasting glucose.

The recent revised classification of diabetes includes four clinical classes:
• type 1 diabetes (results from β-cell destruction, usually leading to absolute insulin deficiency)

• type 2 diabetes (results from a progressive insulin secretory defect on the background of insulin resistance)

• other specific types of diabetes due to other causes, e.g., genetic defects in β-cell function, genetic defects in insulin action, diseases of the exocrine pancreas (such as cystic fibrosis), and drug or chemical induced (such as in treatment of AIDS or after organ transplantation).

• Gestational diabetes mellitus (GDM) (These include categories of gestational impaired glucose tolerance

• Some patients cannot be clearly classified as type 1 or type 2 diabetes. Clinical presentation and disease progression vary considerably in both types of diabetes.

Occasionally, patients who otherwise have type 2 diabetes may present with ketoacidosis. Similarly, patients with type 1 may have a late onset and slow (but relentless) progression of disease despite having features of autoimmune disease. Such difficulties in diagnosis may occur in children, adolescents, and adults. The true diagnosis may become more obvious over time.

1.4.1 Type 1 diabetes - Previously called as juvenile onset or insulin-dependent diabetes mellitus (IDDM), it results from cellular mediated autoimmune destruction of beta cells of pancreas. In this form of diabetes, the rate of beta cell destruction may be variable being rapid in some individuals (mainly infants and children) and slow in others (adults) (ADA, 2008). To survive, people with type 1 diabetes must have insulin delivered by injection or a pump. This form of diabetes usually strikes children and young adults, although disease onset can occur at any age. This form of diabetes accounts for 5 to 10 percent of all diagnosed cases of diabetes (ADA, 2008).

Risk factors for type 1 diabetes may be autoimmune, genetic, or environmental. No known way to prevent type 1 diabetes exists. Several clinical trials for the prevention of type 1 diabetes are currently in progress or are being planned.
1.4.2 Type 2 diabetes - Type 2 diabetes accounts for about 90 to 95% of all diagnosed cases of diabetes in adults. It was previously referred to as noninsulin dependent diabetes mellitus (NIDDM), or adult onset DM. It usually begins as insulin resistance, a disorder in which the cells do not use insulin properly and usually have relative insulin deficiency. As the need for insulin rises, the pancreas gradually lose its ability to produce it. What starts as an increased insulin production by the pancreas to meet up with the added demand, ends in a progressive reduction over time, in the ability to secrete enough insulin in response to meals. Ultimately, blood sugar levels in the blood fail to get normalized due to insulin resistance at the onset of the disease, Due to insulin insufficiencies, the disease progresses, eventually creating a need for antidiabetic therapy. Ketoacidosis seldom occurs spontaneously in this type of diabetes, when seen it usually arises in association with the stress of another illness such as infection.

This form of diabetes frequently goes undiagnosed for many years because hyperglycemia develops gradually and not severe enough at an earlier stage for a patient to notice diabetes symptoms (ADA, 2008).

The risk of developing this type of diabetes increases with age, obesity and lack of physical activity. It has also been associated with strong genetic predisposition more so then autoimmune form of type 1 diabetes. However the genetics of this form of diabetes are complex and not clearly defined.

Whether it is type 1 or type 2, at present, the disease can only be controlled with the help of pharmaceutical hypoglycemic drugs and insulin therapies, as there is no cure yet. Although there is ongoing research by scientists at a global level to find the ultimate cure in the form of an artificial pancreas and islet cell transplantation, the reality seems to be years away. However, disease management for suffering patients is being eased at present by the introduction of novel and painless insulin delivery methods such as inhalable insulin, transdermal insulin, insulin pumps, pens, and patches. This brings some respite from using painful and cumbersome insulin injections.
### Table 1 Characteristics of type 1 and type 2 diabetes

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Type 1 Diabetes</th>
<th>Type 2 Diabetes</th>
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<tbody>
<tr>
<td>Metabolic feature</td>
<td>Severe lack of Insulin due to destruction of beta cells</td>
<td>Beta cell do not produce sufficient insulin or the insulin that is produced become less effective</td>
</tr>
<tr>
<td>Age of Onset</td>
<td>&lt; 35, occurs in youth but can occur at any stage</td>
<td>&gt; 35, more common in older people</td>
</tr>
<tr>
<td></td>
<td>Diabetes not always present in other family members</td>
<td>Often Diabetes present in other members of the family</td>
</tr>
<tr>
<td>Body habits</td>
<td>Normal/Wasted</td>
<td>Obese</td>
</tr>
<tr>
<td>Insulin Therapy</td>
<td>Responsive</td>
<td>High doses are required</td>
</tr>
<tr>
<td>Insulin secretagogue Drugs</td>
<td>Unresponsive</td>
<td>Responsive</td>
</tr>
<tr>
<td>Statistics</td>
<td>Accounts for 10% of diabetics</td>
<td>Accounts for 90% of all diabetics</td>
</tr>
</tbody>
</table>
1.4.3 Gestational diabetes - Some women have a tendency to develop gestational diabetes, which appears during the mid or late stages of pregnancy. This is caused due to the increasing levels of pregnancy hormones that have a negative influence on insulin or create a shortage of insulin. Although this form of diabetes disappears after delivery, a woman who has had gestational diabetes is more likely to develop type 2 diabetes later in life. Gestational diabetes, which is detrimental to the unborn baby’s health, is usually treated with a meal plan, exercise, and insulin therapies.

1.5 Prevalence of Diabetes

Diabetes is a common health problem worldwide and is one of the most challenging public health problems of 21st century. Based on World Health Organization report the number of people with diabetes is rapidly increasing worldwide and diabetes has become a major public health concern. From 1985 to 2000, the number of people suffering from diabetes increased from 30 million to 150 million. Moreover, it is estimated that by 2010 the total number of people with diabetes is projected to be 221 million and is expected to increase to 300 million by 2025 (King H et al, 1998) as shown in figure 4.

Diabetes mellitus has diverse geographical distribution. The highest incidences have been reported from India, China, and USA (Ramachandran A et al, 2002). International Diabetes federation in its report in 2000 said that type 2 diabetes constitutes about 85-95% of all diabetes in developing countries and account for an even higher percentage in developed countries.

1.6 Prevalence of Diabetes in India

Incident of diabetes is increasing globally but the developing countries showed maximum Increase during the last four years. Majority of diabetic population in India belong to non-insulin dependent diabetes type (Type 2), which comprises over 95% of diabetes in India (Vishvanathan et al, 2001). In 1970 it was reported that the prevalence of diabetes in India was low as compared to the western world, but recent show that India has the world largest diabetic population. The World Health
Table 2  Top ten countries in the world with estimated people with diabetes in 1995 and the expected number of diabetes in 2025 (King et al, 1998)

<table>
<thead>
<tr>
<th>Countries</th>
<th>Diabetes in millions (1995)</th>
<th>Rank</th>
<th>Countries</th>
<th>Diabetes in millions (2025)</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>INDIA</td>
<td>19.4</td>
<td>1</td>
<td>INDIA</td>
<td>57.2</td>
<td>1</td>
</tr>
<tr>
<td>CHINA</td>
<td>16.0</td>
<td>2</td>
<td>CHINA</td>
<td>37.6</td>
<td>2</td>
</tr>
<tr>
<td>UNITED STATES</td>
<td>13.9</td>
<td>3</td>
<td>UNITED STATES</td>
<td>21.9</td>
<td>3</td>
</tr>
<tr>
<td>RUSSIA</td>
<td>8.9</td>
<td>4</td>
<td>RUSSIA</td>
<td>14.5</td>
<td>4</td>
</tr>
<tr>
<td>JAPAN</td>
<td>6.3</td>
<td>5</td>
<td>JAPAN</td>
<td>12.4</td>
<td>5</td>
</tr>
<tr>
<td>BRAZIL</td>
<td>4.9</td>
<td>6</td>
<td>BRAZIL</td>
<td>12.2</td>
<td>6</td>
</tr>
<tr>
<td>INDONESIA</td>
<td>4.5</td>
<td>7</td>
<td>INDONESIA</td>
<td>11.7</td>
<td>7</td>
</tr>
<tr>
<td>PAKISTAN</td>
<td>4.3</td>
<td>8</td>
<td>PAKISTAN</td>
<td>11.6</td>
<td>8</td>
</tr>
<tr>
<td>MEXICO</td>
<td>3.6</td>
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<td>MEXICO</td>
<td>8.8</td>
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</tr>
<tr>
<td>UKRAINE</td>
<td>3.8</td>
<td>10</td>
<td>UKRAINE</td>
<td>6.5</td>
<td>10</td>
</tr>
<tr>
<td>All other</td>
<td>49.7</td>
<td>-</td>
<td>All other Countries</td>
<td>103.6</td>
<td>-</td>
</tr>
</tbody>
</table>
**Figure 3** Estimated increase in numbers of diabetics worldwide from year 1985 to 2025 (King H et al, 1998)
Organization has estimated that in 1995, 19.4 million people were affected by diabetes in India. Today India has more than 25 million diabetic patients more than any other countries in the world and this number is expected to rise to over 35 million by 2010 and is expected to increase to 52.2 million by year 2025, which will be equal to one sixth of world total diabetic population (King H et al, 1998; Ramachandran A et al, 2003).

According to present trend regarding increase in diabetes, it has been estimated that by the year 2040 the diabetic population in India may reach up to 80.9 million (Bjork S et al, 2003).

The prevalence of diabetes is higher in urban India as compared to rural population. This striking four to six fold lower prevalence of diabetes in rural India is attributed to continuation of traditional Indian life style, which has its beneficial effect on glucose tolerance. Although the prevalence of diabetes is lower in rural India and since two third of Indian population lives in rural India, the prevalence of diabetes in urban areas is more prominent and is a cause of concern, due to change in dietary habits, low physical activities, sedentary lifestyle and socio economic changes etc.

1.7 Factors Responsible for Susceptibility towards Diabetes

The important factors contributing to risk for high prevalence of diabetes includes (1) Genetic predisposition (2) Insulin Resistance (3) Obesity (4) Rabid Urbanization (5) Aging population

1.7.1 Genetic factors - Researchers while studying identical twins and the family tree of patients with diabetes have found that hereditary is an important factor contributing to the development of type 2 diabetes. A positive family history confers 2-4 fold increase risk of type 2 diabetes. It may be possible to test family members to determine their risk of developing the condition. It has been estimated that 2-5 % patients with type 2 diabetes may have MODY forms of diabetes. Studies have indicated that insulin resistance is more pronounced in MODY compared to classical adult onset Indian type 2 diabetic subjects (Mohan V et al, 1985a; Mohan V et al, 1985b).
1.7.2 Insulin Resistance - Insulin resistance is an important clinical issue in patients with diabetes particularly in type 2 diabetes. However, its presence may be less evident in patients who are not obese. In a study carried by Mohan V, 2004 evidences high prevalence rate of type 2 diabetes was found in Asian Indians, which was attributed to the higher degree of insulin resistance, compared to the Caucasians (Chandalia M et al, 1999; Mishra A et al, 2002). It was further demonstrated that Asian Indians have higher insulin levels to a glucose load compared to Europeans.

Insulin resistance occurs because of defective insulin mediated glucose uptake and utilization, which reflects the inhibition of glucose transport. Insulin resistance is the major factor that contributes to hyperglycemia. Cross sectional studies carried on first degree relatives of type 2 diabetic patients predicted increased insulin resistance (Herlihy OM et al, 2002) could be a cause of early hyperglycemia and risk factors.

1.7.3 Obesity - Insulin resistance is strongly associated with obesity and physical inactivity, and several mechanisms mediating this interaction have been identified. A number of circulating hormones, cytokines, and metabolic fuels, such as non-esterified (free) fatty acids (NEFA) originate in the adipose tissue and modulate insulin action. An increased mass of stored triglyceride, especially in visceral or deep subcutaneous adipose depots, leads to large adiposities that are themselves resistant to the ability of insulin to suppress lipolysis. This result in increased release and circulating levels of NEFA and glycerol, both of which aggravate insulin resistance in skeletal muscle and liver.

Patients with insulin resistance have a group of related clinical findings: glucose intolerance, central obesity, dyslipidemia, hypertension, and altered fibrinolysis.

The role of obesity in pathogenesis of diabetes is complex and confounded by many heterogeneous factors. Indeed the relationship between diabetes and obesity has given the term diabesity to characterize the close association between these two disorders. Another study by Singh RB et al, 1998 showed that overweight obesity and the central obesity were significantly associated with diabetes. Obesity has been on the increase in children, which might play a causative role in the escalating prevalence of diabetes in the young (Ehtisham S et al, 2004; Bloomgarden, 2002). This increased occurrence of overweight in childhood may be the first sign of insulin resistance and future metabolic syndrome.
Figure 4 Prevalence level of Diabetes Mellitus in developed and developing countries from 1995 to that estimated in 2025
1.7.3.1 Central Obesity – The android pattern of the body fat typified by more upper body adiposity measured as waist hip ratio (WHR) has been found to be a greater risk factor for type 2 diabetes than the general adiposity (Ramachandran A et al, 2002). Studies have shown that central obesity is common in Indians despite low rates of obesity. The adverse effect of central obesity is manifested in increasing tertiles of BMI both in men and women. The effect being more evident in the women. This is probably one of the reasons for the higher prevalence of diabetes in women in urban area.

1.7.4 Rapid Urbanization- Urbanization has brought about marked variation in the living condition. Socio-economic development over the last 40-50 years has resulted in dramatic changes in the lifestyle from traditional to modern. This has resulted in physical inactivity due to technological advancement where most of the manual work has been supplemented by modern gadgets. In the urban area there are wide social and economic disparities. It has also been demonstrated that prevalence of diabetes is lower in the low socio-economic group living in the urban areas compared with those belonging to the high-income group (12.6 vs. 24.6%) (Ramachandran A et al, 2002). This was probably related to the physical activity of the low-income group as most of them were involved in some of the physical exercise during their work time. Therefore prevalence of diabetes and increased glucose tolerance is found to be lower in the low-income group significantly. This indicates that the sedentary life style among the high-income group accompanies diabetes. In addition to sedentary life style, dietary pattern and high level of mental stress also effect insulin sensitivity, fuelling obesity leading to diabetes.

1.7.5 Aging Population - One of the most predictable and universal biochemical changes that occur, as we grow old is a progressive loss of glucose tolerance, characterized by prolonged post-meal elevations of glucose and insulin. As glucose tolerance continues to worsen, it increases our risk for obesity, hypertension, coronary artery disease and diabetes.
1.8 Complications of Diabetes Mellitus

Diabetic complications are the major cause of morbidity and mortality in diabetic patients. Chronic hyperglycemia is the major initiator of diabetic micro vascular complications (e.g. retinopathy, neuropathy and nephropathy). Glucose processing uses variety of diverse metabolic pathway; chronic hyperglycemia can induce multiple cellular changes leading to complications. Two landmark studies conducted by The Diabetes Control and Complication Trial (DCCT), 1993 and the United Kingdom Prospective Diabetes Study (UKPDS), 1998 showed that intensive control of hyperglycemia could reduce the occurrence or progression of retinopathy, neuropathy and nephropathy in persons with type 2 diabetes. Several predominant well-researched theories have been proposed to explain how hyperglycemia can produce the neuronal and vascular derangements that are hallmarks of diabetes. These theories can be separated into those that emphasize the toxic effect of hyperglycemia and its pathophysiological derivatives (such as oxidants, hyperosmolarity and glycation products) on tissues directly and those that ascribe pathophysiological importance to the sustained alteration in cell signaling pathway, such as changes in phospholipids or kinases induced by products of glucose metabolism (Bursell SE et al, 1996).

All the mechanisms which have been implicated in glucose mediated vascular damage seem to reflect that hyperglycemia induces processes leading to overproduction of superoxide by mitochondrial electron transport chain. This integrating paradigm provides a new conceptual framework for future research and drug discovery.

1.8.1 Diabetic retinopathy - Diabetic Retinopathy is deterioration of small blood vessels that nourish retina. This involves narrowing, hardening, bulging, hemorrhaging of several veins and capillaries of retina. This is a serious complication and may lead to loss of vision, contraction of the visual field, changes in size of the object or photophobia.

Regional ischemia appears to be a central process in the development of diabetic retinopathy (Kohner EM, 1976; Kohner EM, Oakley NW, 1975). Initially, a compensatory increase in both volume and segment retinal blood flow occurs, with
auto regulatory dilatation of retinal vessels (Kohner EM, 1976; Cunha-Vaz JG et al, 1978).

Diabetic retinopathy is a highly specific vascular complication of both types of diabetes. Hyperglycemia induces metabolic disorders that initiate a sequence of events that lead to retinopathy (Kowluru RA et al, 2001). Multiple hypotheses have been proposed to account for hyperglycemia-induced retinopathy including sorbitol pathway hyperactivity (Kador PF, 1988), nonenzymatic glycation resulting in an accumulation of AGEs (Hammes HP et al, 1999), oxidative stress (Kowluru RA et al, 1996) and protein kinase C activation (Aiello LP et al, 1997). ROS has been involved in decreased retinal blood flow (Bursell SE et al, 1997), increased vascular permeability and disruption of blood-retinal barrier (Ellis EA et al, 1998; Ellis EA et al, 2000) and the appearance of acellular capillaries from the apoptotic loss of retinal capillary cells (Hammes HP et al, 1997; Kern TS et al, 2000). In the more advanced stage, termed 'proliferative retinopathy', hemorrhages, retinal detachment and other serious forms of deterioration are observed. When the disease progresses to this late stage total blindness may occur. It usually takes between 10-13 years for diabetic retinopathy to develop and it is present in some degree in most diabetics who have had the disease for more than 20 years. In only about half of the diabetics who develop retinopathy the vision is markedly impaired and total blindness occurs in only about 6%. Still, diabetes is the leading cause of blindness in adults and is estimated to cause from 12,000 to 24,000 new cases each year. Two other complications of diabetes, cataracts and glaucoma, can also lead to loss of vision. The development of laser therapy has reduced the prevalence of diabetes-induced blindness; however this therapy is not without occasional side effects (hemorrhage, retinal detachment and loss of visual field) and is therefore indicated only for the more serious conditions.

**Cataracts** - are clouding of the normally clear lens. A cataract develops over years and causes blurred vision when a large part of the lens becomes cloudy. Causes of cataracts include aging, eye injuries, disease, heredity, and birth defects. Cataracts can be treated by surgical removal of the lens. Eyeglasses, contact lenses, or intraocular lens implants restore vision following surgery.
1.8.2 Diabetic nephropathy affecting kidneys - It is a complication of chronic diabetes. Nephropathy is less frequent than retinopathy and develops with long standing diabetes when glucose levels are poorly controlled. According to statistical predictions, out of million patients with diabetes in India, diabetic nephropathy is expected to develop in 6.6 million (Hossain P et al, 2007). Nephropathy results from damage to the bundle of capillaries, which are involved in the formation of kidney filtering system. An early manifestation of diabetic nephropathy is microalbuminuria, which is defined as elevated urinary albumin excretion below the level of clinical albuminuria. Kidney functions tests help determine the degree of kidney damage. Treatments for kidney failure include hemodialysis, peritoneal dialysis and kidney transplants.

1.8.3 Diabetic neuropathy affecting nerves - Diabetic neuropathies are among the most frequent complication of long-term diabetes. It is estimated that 60% to 70% of diabetics have mild to severe forms of nervous system damage. Neuropathy occurs in people with Type II diabetes due to metabolic changes with diabetes. Constant high blood sugar destroys both nerve fiber (axon) and the fatty insulation

<table>
<thead>
<tr>
<th>COMPLICATIONS</th>
<th>ORGAN AFFECTED</th>
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<tr>
<td>Coronary Heart Disease</td>
<td>Heart</td>
</tr>
<tr>
<td>Dermopathy</td>
<td>Skin</td>
</tr>
<tr>
<td>Microangiopathy</td>
<td>Small Blood Vessels</td>
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<tr>
<td>Macroangiopathy</td>
<td>Large Blood Vessels</td>
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<tr>
<td>Nephropathy</td>
<td>Kidney</td>
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<tr>
<td>Neuropathy</td>
<td>Nerves</td>
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<tr>
<td>Peripheral Vascular Disease</td>
<td>Blood vessels of Legs and Feets</td>
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<tr>
<td>Retinopathy</td>
<td>Eyes</td>
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that surrounds it (myelin). Damaged nerves do not transmit proper signals, resulting in a loss of sensation, hyper sensation, or pain.

Peripheral neuropathy is the most common form. It can be described in patients with primary (type 1 and 2) and secondary diabetes of diverse causes suggesting a common etiologic mechanism based on chronic hyperglycemia. Varying from mild to severe, it causes changes in sensation that begin in the toes move up to the feet and legs. One may experience numbness, tingling, burning, dull ache, or stabbing pain and cramping, which is worse at night. The skin can become so sensitive that pressure from clothes is painful. Severe neuropathy may cause weakness and unbalanced walking. The greatest danger is foot ulcers, which result when lack of sensation causes people to continue walking on injured feet. Autonomic neuropathy involves the nerve supply to small blood vessels and sweat glands of the skin, the stomach, the bladder, the heart, and the nervous system etc. It is most often associated with long-term diabetes, poor control, and elevated blood sugar.

1.8.4 Macrovascular complications affecting blood vessels - Cardiovascular disease is the leading cause of mortality among diabetics (Caprio S et al, 1997). The blood vessel wall thickens, becomes hard and non elastic (called as Arteriosclerosis), blood vessels also becomes clogged with mounds of plague (known as Atherosclerosis). Eventually the flow of blood may be blocked. Three types of this disease are recognized:

**Peripheral Vascular Disease** – Arises from abnormality in blood vessels that supply the blood to legs and feet. This can cause weakness and pain while walking in the initial stage and when the artery wall is completely blocked it causes severe pain and the legs becomes cold and pale. Treatment includes replacing the diseased artery surgically or opening the blood vessels against artery wall.

**Coronary Artery Disease** – This disease deals with diseased heart arteries. Cramping and angina may occur when the flow of blood is decreased and complete blockage results in myocardial infarction leading to heart attack. Treatments include coronary bypass surgery. The development of coronary artery diseases is the most common macrovascular complication of diabetes particularly of type 2 diabetes (Kennel WB et al, 1990; Laakso M et al, 1995).
Cerebral Vascular Disease – Non fatal or small infarction, especially with multiple occurrences, is a feature of cerebrovascular disease, complicating diabetes mellitus. Temporal reduction in the supply of blood may cause partial blockage while a complete loss of blood supply to the area of brain due to clogging result in cerebrovascular stroke.

1.9 Mechanism of Hyperglycemia induced Diabetic Complications

Many hypotheses about how hyperglycemia causes diabetic complications have generated large amount of data as well as several clinical trials based on specific inhibitors of these mechanisms. The mechanisms underlying the development of diabetic late complications have been reviewed by Brownlee M et al, 2001. The major theories are as follows: Aldose Reductase theory, Advanced Glycation End Product (AGE) Theory, Activation of Protein Kinase C (PKC) Isoform Theory, and Increase in Hexosamine Pathway Flux. In addition Reactive Oxygen Intermediate Theory has also been found to be responsible for diabetes complications.

1.9.1 Aldose Reductase - It is the first enzyme in the polyol pathway. It is a cytosolic, monomeric oxidoreductase that catalyses the NADPH dependent reduction of glucose. Increased intracellular glucose in hyperglycemic environment results in its increased enzymatic conversion to the alcohol sorbitol, with concomitant decrease in NADPH. In the polyol pathway, sorbitol is oxidized to fructose by the enzyme sorbitol dehydrogenase, with NAD⁺ being reduced to NADH. Cataract formation in diabetes and galactosemia results from accumulation in the lens of excessive sorbitol synthesized by the action of aldose reductase on glucose or galactose.

1.9.1.1 Polyol Pathway - The polyol pathway is a two-step metabolic pathway in which glucose is reduced to sorbitol, which is then converted to fructose. It is one of the most attractive candidate mechanisms to explain, at least in part, the cellular toxicity of diabetic hyperglycemia because (i) it becomes active when intracellular glucose concentrations are elevated, (ii) the two enzymes are present in human tissues and organs that are sites of diabetic complications, and (iii) the products of the
pathway and the altered balance of cofactors generate the types of cellular stress that occur at the sites of diabetic complications.

A number of mechanisms have been proposed to explain the potential detrimental effects of hyperglycemia-induced increase in polyol pathway flux. These include sorbitol induced osmotic stress, decreased (Na\(^+\), K\(^+\)) ATPase activity, an increase in cytosolic NADH/NAD\(^+\) and a decrease in cytosolic NADPH. Hyperglycemia induced activation of PKC increases cytosolic phospholipase A2 activity, which increases the production of two inhibitors of Na\(^+\) K\(^+\) ATPase, arachidonate and PGE2. It has also been proposed that reduction of glucose to sorbitol consumes NADPH. Since NADPH is required for regenerating reduced glutathione (GSH) this could induce or exacerbate intracellular oxidative stress.

1.9.2 Advanced Glycation End Products - Advance glycation end product is a class of complex products. They are the results of reaction between carbohydrates and free amino group of proteins. The AGEs are infact the result of glycoxidation but as shown recently may be an end product of lipid peroxidation (Fu MX \textit{et al}, 1996; Niwa T \textit{et al}, 1997).

AGEs may be formed external to the body (exogenously) by heating (e.g. cooking) sugars with fats or proteins (Koschinsky T He CJ \textit{et al}, 1997) or, inside the body (endogenously) through normal metabolism and aging. Under certain pathologic conditions (e.g. oxidative stress due to hyperglycemia in patients with diabetes), AGE formation can be increased exceeding normal levels.

In the pathogenesis of diabetes-related AGE formation, hyperglycemia results in higher cellular glucose levels in those cells unable to reduce glucose intake (e.g. endothelial cells) (Dominiczak MH, 2003; Brownlee M, 2005). This in turn results in increased levels of NADH and FADH, increasing the proton gradient beyond a particular threshold at which the complex III prevents further increase by stopping the electron transport chain. This results in mitochondrial production of reactive oxygen species, activating PARP1 by damaging DNA. PARP1 in turn, ADP-ribosylates GAPDH, a protein involved in glucose metabolism, leading to its inactivation and an accumulation of metabolites earlier in the metabolism pathway. These metabolites activate multiple pathogenic mechanisms, one of which includes increased production of AGEs.
1.9.3 **Diacylglycerol (DAG) and Protein Kinase C** - These are critical intracellular signaling molecules that can regulate many vascular functions including permeability, vasodilator release, endothelial activation and growth factor signaling. In this pathway, hyperglycemia inside the cell increases the synthesis of a molecule called diacylglycerol, which is a critical activating cofactor for the classic isoforms of protein kinase-C, -β, -δ, and -α. When PKC is activated by intracellular hyperglycemia, it has a variety of effects on gene expression. In each case, the things that are good for normal function are decreased and the things that are bad are increased.

The PKC family comprises at least eleven isoforms, nine of which are activated by lipid second messenger DAG. Intracellular hyperglycemia increases the amount of DAG in cultured micro vascular cells and in the retina and renal glomeruli of diabetic animals (*Koy D and King G, 1998*). Increased de novo synthesis of DAG leads to activation of beta isoforms of PKC, which have been shown to mediate the retinal and renal blood flow abnormalities (*Ishii H et al, 1996*). Activation of PKC by raised glucose also induces expression of permeability enhancing factor VEGF in smooth muscles cells (*Williams B et al, 1997*). Treatment with an inhibitor specific for PKC significantly reduced PKC activity in the retina and renal glomeruli of diabetic animal. Furthermore, the treatment significantly reduces diabetes induced increase in retinal mean circulation time, normalizes increase in glomerular filtration rate and partially corrected urinary albumin excretion.

1.9.4 **Hexosamine Pathway** - When glucose is high inside a cell, most of that glucose is metabolized through glycolysis, going first to glucose-6 phosphate, then fructose-6 phosphate, and then on through the rest of the glycolytic pathway. However, some of that fructose-6-phosphate gets diverted into a signaling pathway in which an enzyme called GFAT (glutamine: fructose-6 phosphate amidotransferase) converts the fructose-6 phosphate to glucosamine-6 phosphate and finally to UDP (uridine diphosphate) N-acetyl glucosamine. What happens after that is the N-acetyl glucosamine gets put onto serine and threonine residues of transcription factors, just like the more familiar process of phosphorylation, and over modification by this glucosamine often results in pathologic changes in gene expression.
The excessive flux of glucose or FFAs into a variety of cell types results in the activation of the hexosamine biosynthetic pathway (Marshall S et al, 1991; Boden et al, 1994), which in turn leads to insulin resistance and the development of late complications of diabetes (Marshall S et al, 1991; Boden G et al, 1994; Schleicher ED & Weigert C, 2000). Researchers have implicated a hyperglycemia-induced increase in ROS formation in the activation of the hexosamine pathway (Brownlee M et al, 2001).

1.10 Oxidative Stress and Diabetes

Each stage of the diabetes continuum, from insulin resistance through impaired glucose tolerance to overt type 2 diabetes, is accompanied by oxidative stress. Reduction of oxygen leads to the generation of oxygen free radicals, which are the ultimate cause of oxidative stress. Reactive oxygen species (ROS) consist of oxygen free radicals and associated entities that include superoxide free radicals, hydrogen peroxide, singlet oxygen, nitric oxide (NO) and peroxynitrite (Chong ZZ et al, 2005). Most species are produced at low levels during normal physiological conditions and re scavenged by endogenous antioxidant system that include superoxide dismutase, glutathione peroxidase, catalase, and small molecule substances such as vitamin C and Vitamin E. Superoxide radical is the most commonly occurring oxygen free radical that produces hydrogen peroxide by dismutation. Other enzymes capable of producing superoxide are xanthine oxidase, NADPH oxidases and cytochrome P450. Superoxide produces hydrogen peroxide through Haber –Weiss reaction in the presence of ferrous ion by manganese (Mn)-SOD or copper (Cu)-SOD. In the presence of transition elements, a reaction of hydrogen peroxide with superoxide results in the formation of hydroxyl radical, the most active oxygen free radical. Alternatively hydroxyl radical may be formed through an interaction between superoxide radical and NO (Fubini B & Hubbard A, 2003). The interaction between nitric oxide and superoxide radical results in the generation of peroxynitrous acid. Hydroxyl radical is produced from spontaneous decomposition of peroxynitrous acid. Both NO itself and peroxynitrite are also recognized as active oxygen free radicals. In addition to directly altering cellular function. NO may work through peroxynitrite that is potentially considered a more potent radical than NO itself (Pfeiffer S et al, 2001).
Oxidative stress represents an important pathway for the destruction of cells (Chong H et al, 2005; Li DH et al, 2006). The production of ROS can lead to cell injury through cell membrane lipid destruction and cleavage of DNA (Vincent AM & Maiese K, 1999; Wang et al, 2003). ROS result in peroxidation of cellular membrane lipids (Siu AW & To CH, 2002). The cleavage of DNA during the hydroxylation of guanine and methylation of cytosine (Lee DH et al, 2002), and the oxidation of proteins that yield protein carbonyl derivatives and nitrotyrosine (Adams S et al, 2001). In addition to the detrimental effects to cellular integrity, ROS can inhibit complex enzymes in the electron transport chain of the mitochondria resulting in the blockade of mitochondrial respiration (Yamamoto T et al, 2002).

The pathogenic effect of hyperglycemia, possibly in concert with free fatty acid release, is mediated to a significant extent via increased production of ROS. In addition to their ability to directly inflict damage on macromolecules, ROS indirectly lead to tissue damage activating a number of cellular stress sensitive pathways, oxidative stress may decrease insulin sensitivity and injure the insulin producing cells within the pancreas. For example ROS can penetrate through cell membranes and cause damage to beta cells of pancreas (Chen H et al, 2005; Lepore DA et al, 2004). In addition free fatty acids, which can lead to ROS, have been shown to also contribute to mitochondrial DNA damage and impaired pancreatic beta cells function (Rachek LI et al, 2006).

Oxidative stress is also believed to modify a number of signaling pathways within a cell that can ultimately lead to insulin resistance. As a result; it is possible that activation of oxidative stress pathways plays a key role in the development of not only the late complications in type 1 and type 2 diabetes mellitus but also insulin resistance.

A number of oxidative stress pathways; responsible for insulin resistance can be highlighted in non-diabetic rats. Hyperglycemia was shown to lead to a significant decrease insulin stimulated glucose uptake, a significant increase in muscle protein carbonyl content (used as an indicator of oxidative stress), and elevated levels of malondialdehyde and 4-hydroxynonenal as an indicator of lipid peroxidation (Haber CA et al, 2003). These biological markers of oxidative stress and insulin resistance were normalized during the application of antioxidant N-acetylcysteine or taurine to suggest that oxidative stress contributes to the pathogenesis of hyperglycemia induced
insulin resistance (Haber CA et al, 2003). Furthermore hyperglycemia can lead to increased production of ROS in endothelial cells, liver, pancreatic beta cells.

![Figure 5 Reactive oxygen species](image)

There are some evidences, which have indicated the role of oxidative stress in development and progression towards diabetes. It has recently been shown that there is a correlation between iron stores and susceptibility to type 2 diabetes. The implication is that raised levels of redox active metals lead to raised level of reactive oxygen species and increased probability of diabetes

1.11 Heredity Factors and Genetic Aspect of Diabetes

There are various factors that cause or exacerbate diabetes mellitus. Among them genetic background is considered to be an important one. Talking about type 2 diabetes, it is one among the complex diseases of which genetic contribution is well accepted. Despite of diverse phenotypic nature of type 2 diabetes, twin family history studies, and the wide spectrum of diabetes prevalence across populations provide convincing evidence for an important role of genetic studies in development of type 2 diabetic syndrome.

The concordance rate among the identical twins may approach 100 % (Pyke DA et al, 1976). Concordance increases with duration of follow up, but even more conservative estimates place long-term concordance at nearly 60 % (Newman B et al, 1987). This concordance rate is at least double that of dizygotic twins or siblings
whose lifetime risk has been estimated at approximately 25-38% (Newman B et al, 1987; Poulsen P et al, 1999). Such figures are consistent with major genetic effect. Although the polygenic nature of disease has made it difficult to dissect out individual gene conferring increased risk for diabetes.

The role of genetic susceptibility in causal of complex diseases can be seen by measuring the ratio of the risk of an unaffected relative of an affected (diabetic) individual to the of general or control population (Risch N, 1990). The family member used is most often a sibling, thus providing parameters λS which is the ratio of risk of the sibling of T2DM individual to that of general population.

Before the onset of fully developed type 2 diabetes, individuals at risk of type 2 diabetes mellitus show impaired insulin action (Warram JH et al, 1990) and impaired insulin secretion (Pimenta W et al, 1995). Evidences now suggest that both defects proceed and predict late type 2 diabetes (Weyer C et al, 1999) and that both defects are inherited (Bogardus C et al, 1989; Schumacher MC et al, 1992; Sakul H et al, 1997; Elbein SC et al, 1999).

Several studies carried on diabetic families have pointed that Indians have a genetic predisposition to diabetes, which gets easily unmasked when the environmental conditions are adverse. This shows that both nature and nurture plays important role in development of the disease. The fact that 75% of type 2 diabetic patients have first-degree family history of diabetes indicates a strong familial aggregation in Indian diabetic patients (Ramachandran C et al, 2000). This study confirms the utility of family history as a public health tool for risk determination and prevention of diabetes.

1.11.1 Type 2 Diabetes Mellitus is a Genetic Disease: Classical Evidence—Various studies have been carried out for finding out the genetic basis of diabetes:
1.11.1.1 The spectrum of T2DM in different ethnic groups

The prevalence of T2DM varies widely among the populations, from 1% in Chile Mapuche Indian, 2% among Caucasians in Europe to as high as 41% in Nauru (Pacific Island) and 50% among Pima Indians in Arizona (Diamond J, 2003). Part of this observed ethnic variability can be attributed to non genetic environmental and cultural factors, however the observation that the disease prevalence varies substantially among ethnic groups that share a similar environment supports the idea that genetic factors contributes to disease predisposition.

1.11.1.2 Familial Aggregation

Other than genes, families share environments, culture and habits. Yet familial aggregation of the disease is another source of evidence for a genetic contribution to the disease. Evidence for a genetic role includes the nearly 4-fold increase risk for T2DM in siblings of a diabetic proband compared with the general population, the odds ratio or of 3.4-3.5 with only a single affected parent, and the increase is to 6.1 if both parents are affected (Meigs JB et al, 2000).

1.11.1.3 Twin Studies

Multiple studies of twin concordance rates have been undertaken in T2DM. Estimates for concordance rates have been ranged from 0.29 to 1.00 in monozygotic (MZ) twin. While in dizygotic (DZ) twin the range was 0.10-0.43 (Barnett AH, 1981; Newman B et al, 1987; Poulsen P et al, 1999; Medici F et al, 1999). Concordance between both MZ and DZ twin increase with the duration of follow up period (Medici F et al, 1999). In spite of several caveats in twin studies, the high concordance in MZ twins and the 50% fall in DZ twins provides compelling evidence for a genetic component of type 2 diabetes.

1.11.1.4 Habitability of intermediate Phenotype

Insulin sensitivity and insulin secretion deteriorate in parallel in most human T2DM. Both defects predicted subsequent T2DM in several studies and both defects are shown to be present in non diabetic but genetically identical co-twins of a diabetic proband (Vaag et al, 1995). Data from multiple laboratories support a genetic basis for measures of both insulin sensitivity and insulin secretion (Elbein SC et al, 1999; Elbein SC et al, 2000; Gerich JE, 1998).
1.12 Red Blood Cells - Model of Study

Red blood cells along with its membrane have always been an important medium for the study due to important role it plays in varied physiological and metabolic aspects. They are few kinds of cell in the body with no nucleus and only a thin layer of protein skeleton under their membrane, they are living bags of hemoglobin. Erythrocyte has been increasingly studied because it is the easiest available human cell type. The erythrocytes have been modified by evolutionary forces into a highly specialized tissue transports oxygen from lung to the tissues and partially excrete carbon di oxide from waste.

Red blood cells are capable of extreme changes in the shape. Due to their flexibility, red blood cells can easily squeeze through capillaries much narrower than their diameter and can recover rapidly to their original shape. Mature blood cells is biconcave disk shaped with a diameter of 8 micron having an average life span of 120±20 days. Due to absence of cell organelles particularly nucleus and mitochondria, red blood cells loses its ability to synthesize amino acids and fatty acids as such red blood cells have a limited capacity of metabolism barely enough to survive its life span. Red blood cells are continuously being removed from the circulation and to maintain their adequate number they are regularly being formed from the bone marrow in accordance with their removal from reticuloendothelial cell.

Mature red blood cells due to loss of its synthetic capacity are unable to replace enzymes, repair its membrane and utilize oxygen as a source of energy. Red blood cells maintain their physiological state through the supply of energy in the form of ATP formed exclusively through the breakdown of glucose. Red blood cells also has the capacity for NAD+/NADP+ reduction and glutathione synthesis (GSH) along with formation of 2,4 DPG. The formation of reduced glutathione enables red cells to protect -SH group of proteins against oxidation and to trap metallic ions by forming mercantile.

Erythrocyte has an array of endogenous antioxidants involved in quenching oxidant production and exponential chain reaction in diabetes. When the erythrocyte is oxidatively stressed, the risk of diabetes and its progression is increased.

In erythrocytes the group of antioxidants includes Catalase, GSH, GPx, metHb reductase, NADH and SOD. Catalase, SOD, Gpx is central to erythrocyte antioxidant
function. Erythrocytes have also been reported to play a crucial role in recycling ASC in blood plasma (Mendiratta ZC et al, 1998).

Alteration in blood rheological properties has been reported in diabetes mellitus. Changes in lipid composition of red blood cell (RBC) membranes resulting in an impairment of RBC deformability may play a role in altered blood rheological pattern, which plays causative role in pathogenesis of diabetic complications.

Screening that includes erythrocyte oxidative stress determination may provide an additional marker in both preclinical and advanced disease. Several studies have been carried out on erythrocyte of type 2 diabetic patients to find out the marker of oxidative stress.

1.12.1 Red cell membrane

Red cell component is surrounded by a limiting membrane that keep it separated from the extra cellular environment, normal functioning of this membrane is indispensable for the survival of red cells in blood circulation. A disorder of membrane functions by virtue of its role in determining cell size, shape and deformability characteristics and/or disorder in the normal function of the cell membrane properties due to change in cell shape, lead to diminished red cell survival. Deformability of the membrane due to ATP depletion or due to change in membrane, associated with hemoglobin in the cell has its influence on the deformability and the rheological properties of the cell.

Although the erythrocytes are highly deformable and can readily alter its shape in a constant area, but at the same time this limiting membrane of RBC is highly resistant to dilation thereby accounting for the degree of resistance to hypotonic osmotic lysis.

Various alteration of red blood cells (RBC) plasma membrane appears in diabetes mellitus. Diabetes mellitus decreases RBC life span, therefore it may change the plasma membrane by acting through its effect on the ageing process.
Figure 6- Structure of Red blood cells
It has been studied that erythrocyte lipid peroxides levels are increased in diabetes and that high lipid peroxides in diabetes may result in destruction of erythrocyte membrane lipids. (Uzel N et al, 1987)