Introduction

Diabetes mellitus is one of the most common chronic diseases in nearly all the countries. The member of diabetics continues to increase in number and significance day by day because of the changing lifestyles. Estimates of the current and future burden of diabetes are important in order to allocate community and health resources, and to emphasise the role of lifestyle, and encourage measures to counteract the trends leading to the increasing prevalence (Shaw et al., 2010).

Diabetes mellitus can be considered as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient. It is often not very clear which abnormality, if either alone or in combination is the primary cause of the hyperglycemia.

The world prevalence of diabetes among adults (aged 20-79 years) was 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7% and 439 million adults by 2030. Between 2010 and 2030, there will be a 69% increase in number of adults with diabetes in developing countries and a 20% increase in developed countries (Shaw et al., 2010). World Health Organization (WHO) has
predicted that India would experience the largest increase in type 2 diabetes and would have the greatest number of diabetic individuals in the World by the year 2030 (31.7 million in 2000 to 79.4 million in 2030). In India, diabetes among the adult urban populations varies from a low of 5.4% in a northern state to a high of 12.3–15.5% in Chennai, South India, and 12.3–16.8% in Jaipur, Central India (Gupta and Misra, 2007).

Diagnostic criteria for diabetes mellitus

For decades, the diagnosis of diabetes has been based on glucose criteria, either the Fasting plasma glucose (FPG) or the 75-g Oral glucose tolerance test (OGTT). In 1997, the first Expert Committee on the Diagnosis and Classification of Diabetes Mellitus revised the diagnostic criteria, using the observed association between the FPG levels and the presence of retinopathy as the key factor with which to identify threshold glucose levels. The Committee examined the data from three cross-sectional epidemiologic studies that assessed retinopathy with fundus photography or by direct ophthalmoscopy and measured glycemia as FPG, 2-h plasma glucose and A_1C. These studies demonstrated glycemic levels below which there was little prevalence of retinopathy and above which the prevalence of retinopathy increased in an apparently linear fashion. Moreover, the glycemic values above which retinopathy increased were similar among the various populations. These analyses were helpful to find out inform a new diagnostic cut point of ≥126 mg/dl (7.0 mmol/l) for FPG and confirmed the long-standing diagnostic 2-h plasma glucose value of ≥200 mg/dl (11.1 mmol/l). A_1C is a widely used marker of chronic glycemia, reflecting average blood glucose levels over a 2- to 3-month period of time. The test plays a critical role in the management of the patient with diabetes, since it correlates well with both microvascular and, to a lesser extent, macrovascular complications and is widely used as the standard biomarker for the adequacy of glycemic management.
There are four methods to diagnose diabetes are as follows

1. $A_1C \geq 6.5\%$. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the Diabetes Control and Complications Trial (DCCT) assay.*

2. $FPG \geq 126\, \text{mg/dl} \,(7.0\, \text{mmol/l})$. Fasting is defined as no caloric intake for at least $8\, \text{h}$. *

3. $2$-h plasma glucose $\geq 200\, \text{mg/dl} \,(11.1\, \text{mmol/l})$ during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of $75\, \text{g}$ anhydrous glucose dissolved in water.*

4. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose $\geq 200\, \text{mg/dl} \,(11.1\, \text{mmol/l})$. *In the absence of unequivocal hyperglycemia, criteria 1–3 should be confirmed by repeat testing.

Classification

Recent advances in the understanding of the etiology and pathogenesis of diabetes have led to a revised classification. Although all forms of diabetes are characterized by hyperglycemia, the pathogenetic mechanism by which hyperglycemia arises differ widely. Some forms of diabetes are characterized by an absolute insulin deficiency or a genetic defect leading to defective insulin secretion, whereas the other forms share insulin resistance as their underlying etiology. Recent changes in the classification reflect an effort to classify diabetes mellitus on the basis of the pathogenetic process that leads to hyperglycemia, as opposed to criteria such as the age of onset or type of therapy etc. (American Diabetes Association, 2005).
Type 1 or Insulin dependent diabetes mellitus (IDDM)

Type 1 diabetes, also referred to as insulin-dependent diabetes mellitus (IDDM), accounts for 5-10% of all cases of diabetes and is due to primarily autoimmune destruction of β-cells of the islets of Langerhans of the pancreas. This results in insufficient insulin production to regulate blood glucose levels, resulting in hyperglycemia.

A) Autoimmune diabetes mellitus

Type 1 diabetes is an autoimmune disease. An autoimmune disease results when the body’s system for fighting infection (the immune system) turns against a part of the body. In diabetes, the immune system attacks and destroys the insulin-producing β-cells in the pancreas (Atkinson and Maclaren, 1994). The pancreas then produces a little or no insulin. A person who has type 1 diabetes must take insulin daily to live. At present, scientists do not know what exactly causes the body’s immune system to attack the β-cells, but they believe that autoimmune, genetic, and environmental factors, possibly viruses, are involved. The rate of destruction is quite variable, being rapid in some individuals and slow in others (Zimmet et al., 1994).

B) Idiopathic

This form of diabetes is also characterized by deficiency as well as a tendency to develop ketosis. However, individuals with idiopathic diabetes lack immunologic markers, which is an indicator of autoimmune destructive process of the β-cells. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity (McLarty et al., 1990). Although only a minority of patients with type 1 diabetes falls into this category, individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes.
Type 2 or Non-Insulin dependent diabetes mellitus (NIDDM)

Type 2 diabetes, formerly called adult-onset diabetes, is the most common form. This form of diabetes usually begins with insulin resistance, a condition in which muscle, liver, and fat cells do not use insulin properly. As a result, the body needs more insulin to help glucose enter cells to be used for energy. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity (Zimmet, 1997; Harris et al., 1995).

Other specific types

Other specific types are currently less common causes of diabetes mellitus, but are those in which the underlying defect or disease process can be identified in a relatively specific manner.

A) Genetic defects of β-cell function

Several forms of diabetes are associated with monogenetic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before the age of 25 years). They are referred to as maturity onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action.

B) Genetic defects in insulin action

There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to symptomatic diabetes (Taylor, 1992).

C) Diseases of the exocrine pancreas

Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With the exception of that caused by cancer, damage to the
pancreas must be extensive for diabetes to occur; adrenocarcinomas that involve only a small portion of the pancreas has been associated with diabetes. This implies a mechanism other than simple reduction in β-cell mass.

D) Endocrinopathies

Several hormones (e.g., growth hormone, cortisol, glucagon, and epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing’s syndrome, glucagonoma, pheochromocytoma, respectively) can cause diabetes. This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically get resolved when the hormone excess is resolved.

E) Drug- or chemical-induced diabetes

Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may induce diabetes in individuals with insulin resistance. Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β-cells (Esposti et al., 1996). There are also many drugs that can impair insulin action Eg. alloxan and streptozotocin (STZ).

F) Infections

Certain viruses have been associated with β-cell destruction. Diabetes occurs in patients with congenital rubella, although most of these patients have HLA and immune markers characteristic of type 1 diabetes. In addition, coxsackievirus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the disease (Pak et al., 1988).

G) Other genetic syndromes associated with diabetes

Many genetic syndromes are accompanied by an increased incidence of diabetes mellitus. These include the chromosomal abnormalities of Down’s
syndrome, Klinefelter’s syndrome and Turner’s syndrome. Wolfram’s syndrome is an autosomal recessive disorder characterized by insulin-deficient diabetes and the absence of β-cells at autopsy (Barrett et al., 1995).

**Gestational diabetes mellitus (GDM)**

Gestational diabetes is characterized by carbohydrate intolerance resulting in hyperglycemia of variable severity with the onset or first recognition during pregnancy. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy (Alessandro et al., 1999). GDM is associated with an increased risk of foetal complications including macrosomia, neonatal hypoglycemia and an increased vulnerability of progeny to develop obesity and diabetes in the later life. Moreover, there is a greater risk of the mother developing type 2 diabetes in later years.

**Symptoms of diabetes mellitus**

- Symptoms of marked hyperglycemia include polyuria, polydypsia, weight loss, sometimes with polyphagia (Cooke and Plotnick, 2008) and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia.

- Other symptoms include fatigue, tingling or numbness in hands or feet, general irritability, dry skin, sores that are slow to heal, nausea, vomiting and stomach pains.

**Pathogenesis of diabetes mellitus**

**Type I diabetes mellitus**

Type 1 diabetes mellitus is a chronic autoimmune disease associated with selective destruction of insulin-producing pancreatic beta cells (Figure 1). The pathogenesis of IDDM is slow but, progressive immunological destruction of the β-cell mass by antigen specific cytotoxic T-lymphocytes augmented by
cytokine release from macrophage and natural killer cells (Atkinson and Eisenbarth, 2001). Despite strong evidence for an association with genetic factors, the concordance rate for type 1 DM is surprisingly low in identical twins. The lack of 100% concordance in identical twins for type I DM has contributed to a search for environmental factors associated with the disease. The pathogenesis of selective β-cell destruction within the islet in type 1 DM is difficult to follow due to marked heterogeneity of the pancreatic lesions.

Figure 1. Pathogenesis of type 1 diabetes mellitus (Potts and Mandleco, 2002)

Type 2 diabetes mellitus

Type 2 diabetes mellitus is a complex disease; both genetic and environmental factors appear to contribute to its development. In most cases, in genetically predisposed individuals, a slow progression from the normal state to impaired glucose tolerance leading to NIDDM has been observed. Both genetic susceptibility and environmental factors contribute to the development of insulin resistance and the impairment of β-cell function that result in insulin deficiency,
leading to impaired glucose tolerance (Figure 2). As the disease progresses, β-cell failure becomes severe, leading to the development of hyperglycemia. Hyperglycemia itself also impairs insulin sensitivity and β-cell function and exacerbates the disease state. Insulin resistance in peripheral target tissues and impaired insulin secretory capacity of pancreatic β-cells contribute to the pathogenesis of type 2 diabetes.

Figure 2. Pathogenesis of type 2 diabetes mellitus (Schmidt et al., 2004)

Complications of diabetes mellitus

The development of micro and macrovascular complications, the occurrence of end-stage renal disease and cardiovascular disease are the major contributors to the excess mortality in type 2 diabetes. Epidemiological studies have confirmed that hyperglycemia is the most important factor in the onset and progression of diabetic complications (Jakus and Rietbrock, 2004). The complications are directly related to blood vessel disease and are generally classified into micro vascular
disease such as those involving the eyes, kidney and nerves and macro vascular
disease concerning the heart and the blood vessels (Plutzky, 2003). The complications
of diabetes mellitus can be divided into two major types

- Acute metabolic complications
- Chronic or Long-term vascular complications

**Acute metabolic complications**

These complications arise within a short time frame due to poor metabolic control.

- Diabetic ketoacidosis (DKA)
- Hyperglycemic hyperosmolar non-ketotic syndrome (HHNS)
- Lactic acidosis (LA)
- Hypoglycemia

**Diabetic ketoacidosis (DKA)**

Diabetic ketoacidosis is an acute, dangerous complication and is always a
medical emergency. DKA is the consequence of absolute or relative insulin
deficiency and concomitant elevation of counter regulatory hormones including
 glucagon, catecholamines, cortisol, and growth hormone, resulting in
hyperglycemia (>200 mg/dL), metabolic acidosis (venous pH <7.3, Bicarbonate
<15 mmol/L), ketosis and varying degrees of dehydration. Increased lipolysis,
with ketone body (β-hydroxybutyrate and acetoacetate) production causes
ketonemia and metabolic acidosis. The ketoacidosis can become severe enough to
cause hypotension and shock. Prompt and proper treatment usually results in full
recovery, though death can result from inadequate treatment, delayed treatment or
from a variety of complications.
Hyperglycemic hyperosmolar non-ketotic syndrome

While not always progressing to coma, this hyperosmolar nonketotic syndrome (HHNS) is another acute problem associated with diabetes mellitus. It has many symptoms in common with diabetic ketoacidosis, but with a different cause, and requires different treatment. In anyone with very high blood glucose levels (usually considered to be above 300 mg/dl or 16 mmol/l), water will be osmotically driven out of cells into the blood.

![Diagram of metabolic pathways in diabetic ketoacidosis and HHNS](Image)

**Figure 3. Mechanisms of diabetic ketoacidosis and HHNS**

The kidneys will also be "dumping" glucose into the urine, resulting in concomitant loss of water, causing an increase in blood osmolality. If the fluid is
not replaced (by mouth or intravenously), the osmotic effect of high glucose levels combined with the loss of water will eventually result in such a high serum osmolality (dehydration) (Figure 3). HHNS is a medical emergency that requires prompt recognition and treatment. Delayed diagnosis and treatment is one of the important factors responsible for the high mortality associated with HHNS (Hollander et al., 2003; Morales and Rosenbloom, 2003).

**Lactic acidosis**

Lactic acidosis is a life-threatening condition characterized by low arterial pH (<7.35) and elevated arterial lactate levels (>5.0 mEq/L). It is the most common metabolic acidosis in humans and is usually a late-stage sequela of serious medical conditions such as sepsis, hypoxia, and cardiac failure. Lactic acidosis occurs regularly, although infrequently, among persons with type 2 diabetes, at rates similar to its occurrence among metformin users (Brown et al., 1998).

**Hypoglycemia**

Hypoglycemia is common in insulin-treated diabetic patients and also occurs occasionally in patients treated with the oral hypoglycemic sulfonylurea agents. Some people with diabetes may develop hypoglycemic event when blood glucose is < 50 mg/dl and some persons with diabetes do not have symptoms even at very low blood glucose. Hypoglycemia may develop if the glucose intake does not match the treatment. The patient may become agitated, sweaty, and have many symptoms of sympathetic activation of the autonomic nervous system resulting in feelings similar to dread and immobilized panic. Consciousness can be altered, or even lost, in extreme cases, leading to coma and/or seizures or even brain damage and death.

**Chronic complications**

Chronic diabetic complications are the main cause of mortality and morbidity associated with diabetes mellitus (Kirpichnikov and Sowers, 2001; Son et al., 2004).
Diabetes provides a distinct model of chronic vascular disease in which altered glucose homeostasis eventuates in multiorgan dysfunction.

- Microvascular- retinopathy, nephropathy and neuropathy
- Macrovascular- coronary artery disease, cerebrovascular disease and peripheral vascular disease
- Combination of micro and macrovascular: diabetic foot and diabetic dermopathy

The damage to small blood vessels leads to a microangiopathy, which causes the following organ-related problems:

**Diabetic retinopathy**

Hyperglycemia induces tissue damage through mitochondrial superoxide production (Brownlee, 2005). The term diabetic retinopathy refers to all vascular changes in the retina occurring in diabetes. Over 135 million individuals are afflicted with diabetes across the world (ADA, 2004). Retinopathy is the most common microvascular complication of diabetes, resulting in growth of friable and poor-quality new blood vessels in the retina as well as macular edema (swelling of the macula), which can lead to severe vision loss or blindness.

**Diabetic neuropathy**

Diabetic neuropathy is a very varied, multifocal disease and is a major cause of morbidity (Stitt et al., 2002). Three major types of neuropathy are distal symmetrical polyneuropathy, focal neuropathy and autonomic neuropathy (King, 2001). Clinical symptoms are related to the site of major involvement, specifically the somatic or autonomic systems. Neuropathy may develop as a result of endothelial dysfunction, which can cause a reduction in neuronal blood flow. Endoneurial capillary density is reduced and correlates with decreased density of myelinated fibres in neuropathic diabetic patients. These effects may impair function of sensory and autonomic neurons in patients with diabetes.
Diabetic nephropathy

Diabetic nephropathy is defined as proteinuria resulting from reversible endovascular damage of the renal filtration capacity by long-standing diabetes mellitus. Diabetic nephropathy is the largest contributor to the total cost of diabetes care worldwide, accounting for approximately 40% of all new patients placed on dialysis therapy (Chiarelli et al., 2003). Prior to the onset of overt proteinuria, specific changes occur in renal functions, causing renal hyperfiltration, hyperperfusion, and increasing capillary permeability to macromolecules. Tyrosine kinase growth factors and diffuse expansion of the mesangial matrix have emerged as logical mechanisms contributing to the etiopathophysiology of diabetic kidney disease (Karl et al., 2005). Diabetes has become the most common single cause of end-stage renal disease in most countries, about 20-30% of patients with type 1 or type 2 diabetes develop evidence of nephropathy, but in type 2 diabetes a smaller fraction of these progresses to end-stage renal disease.

Circulatory and cardiovascular complications of diabetes

Cardiovascular disease is the leading cause of morbidity and mortality among persons with diabetes. The risk factors include hardening of the arteries (arteriosclerosis), deposition of fats (atherosclerosis), low levels of high density lipoprotein-cholesterol (HDL-C), elevated triglycerides and high blood pressure (Jakus and Rietbrock, 2004). Among persons with diabetes several concomitant conditions may affect the aetiology of atherosclerosis: obesity, hyperinsulinemia, abnormalities of platelet function and defects in blood coagulation and flow (Ginsberg, 2000).

Diabetic foot ulcers

Diabetic foot ulcers, is a series of multiple mechanisms, including decreased cell and growth factor response and lead to diminished peripheral blood flow and decreased local angiogenesis, all of which can contribute to
lack of healing in persons with diabetic foot ulcers. Diabetic foot, often due to an overlap between neuropathy and arterial disease, may cause necrosis, infection and gangrene.

**Impaired wound healing**

Wound healing occurs as a cellular response to injury and involves activation of keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets. The physiologic factors include decreased or impaired growth factor production (Galkowska *et al.*, 2006; Goren *et al.*, 2006), angiogenic response (Galiano *et al.*, 2004), macrophage function (Maruyama *et al.*, 2007), keratinocyte and fibroblast migration and proliferation, number of epidermal nerves (Gibran *et al.*, 2002), bone healing, and balance between the accumulation of ECM components and their remodeling by matrix metallo proteins (MMPs) (Lobmann *et al.*, 2002) which contribute to wound healing deficiencies in individuals with diabetes.

**Erectile dysfunction**

Erectile dysfunction is a serious condition that affects 52% of men between the ages of 40 and 70 years. The incidence of erectile dysfunction increases with age, coronary artery disease, peripheral vascular disease, smoking, dyslipidemia, and diabetes mellitus (Feldman *et al.*, 1994). Diabetes has a known pathologic effect on peripheral tissue innervation and vascularization, both of which are critical for erectile function.

**Diabetes and metabolic abnormalities**

Diabetes mellitus is a chronic metabolic disorder of impaired carbohydrates, fat and protein metabolism. It is characterized by hyperglycaemia expressed as abnormal glucose value, which is due to insulin deficiency and/or insulin resistance which results in decreased utilization of carbohydrate and excessive glycogenolysis and gluconeogenesis from amino acid and fatty acids.
Diabetes and carbohydrate metabolism

Carbohydrates from various dietary sources are the primary exogenous source of glucose. Glucose is the main fuel for energy requirement of the body. Therefore, a continuous supply of glucose is necessary to ensure proper function and survival of all organs. The capacity of the liver in the glucose metabolism by glycogen synthesis and glycolysis in the absorptive state are sufficient to account for the clearance of more than one third of the dietary glucose that is absorbed from the gut (Loranne Agius, 2007). Alterations in the carbohydrate metabolism in diabetes are frequently accompanied by changes in the activities of the enzymes that control glycolysis and gluconeogenesis in liver and muscle; in such a way that the latter process becomes favored (Prince and Kamalakkannan, 2006). Insulin suppresses hepatic glucose output by stimulating glycogen synthesis and inhibiting glycogenolysis and gluconeogenesis.

Increased rate of hepatic glucose production results in the development of overt hyperglycemia, especially fasting hyperglycemia, in patients with type 2 diabetes (DeFronzo et al., 1992). Insulin exerts direct effect on the liver (Michael et al., 2000) as well as influences the substrate availability and fluxes of free fatty acids (FFA) (Bergman and Ader, 2000). There are several enzymic checkpoints to control glycolysis (hexokinase, glucokinase), glycogenesis (glycogen synthase kinase-3), glycogenolysis (glycogen phosphorylase) and gluconeogenesis (phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase, glucose-6-phosphatase). The activities of certain enzymes are directly controlled by insulin via phosphorylation and dephosphorylation mechanisms (Zhang, 2002).

Diabetes and lipid metabolism

Diabetes mellitus is a major risk factor for the development of cardiovascular complications and cardiovascular disease accounts for 80% of all diabetic mortality (WHO, 2004). In patients with diabetes, the abnormalities in lipid
metabolism generally lead to elevated levels of serum lipids and lipoproteins that, in turn, play an important role in the occurrence of premature and severe atherosclerosis (Keenoy, 2005; Raanan, 2008). In both type 1 and type 2 diabetes, poor glycemic control increases serum TG levels, VLDL and LDL, and decreases HDL. It is, therefore, important to optimize glycemic control in patients with diabetes because this will have secondary beneficial effects on lipid levels. Excessive lipolysis occurs in diabetic adipose tissue and leads to increase free fatty acids in circulation.

**Diabetes and protein metabolism**

Insulin acts on protein metabolism by increasing the rate of protein synthesis and decreasing the rate of protein degradation. Proteins are an important target for oxidative challenge. Many of the chronic complications of diabetes involve changes in structural proteins.

It is thus possible that changes in protein metabolism are responsible for many of the chronic complications of diabetes mellitus, because even a minor imbalance between protein synthesis and degradation can potentially have a profound effect over the long term on cell viability and metabolism.

**Diabetes and Oxidative stress**

An imbalance between reactive oxygen species (ROS) and the antioxidant defense mechanisms (enzymatic and non-enzymatic) of a cell leads to excessive production of oxygen metabolites, creating a condition frequently termed as ‘oxidative stress’ (Skaper et al., 1997).

The increased blood glucose levels in diabetes produce superoxide anions, which generate hydroxyl radicals via Haber-Weiss reaction, resulting in peroxidation of membrane lipids and protein glycation. This leads to oxidative damage to cell membranes. Therefore, excessive oxidative stress has been
implicated in the pathology and complications of diabetes mellitus (Wolff, 1993). Moreover ROS and RNS can also damage other important biomolecules including carbohydrates, proteins and DNA (Veerapur et al., 2010).

![Diagram of hyperoxidative stress in non-insulin dependent diabetes](Ahmed, 2005)

In boxes are shown mechanisms that are directly related to hyperglycaemia. In circles are some mechanisms that result from the reaction of free radicals (e.g. superoxide $O_2^-$) with lipoproteins (e.g. small, dense low-density lipoprotein, sd LDL) and nitric oxide (NO), ox LDL, oxidized LDL, ONOO- peroxynitrite.

**Figure 4. Pathogenesis of hyperoxidative stress in non-insulin dependent diabetes (Ahmed, 2005)**

In boxes are shown mechanisms that are directly related to hyperglycaemia. In circles are some mechanisms that result from the reaction of free radicals (e.g. superoxide $O_2^-$) with lipoproteins (e.g. small, dense low-density lipoprotein, sd LDL) and nitric oxide (NO), ox LDL, oxidized LDL, ONOO- peroxynitrite.
The primary causal factor is hyperglycaemia and this operates via several mechanisms (Figure 4), although the individual contribution of each mechanism to hyperoxidative stress remains undefined. Glycoxidation of glucose generates reactive oxygen species, such as superoxide, hydrogen peroxide and hydroxyl radical (Giugliano et al., 1996). These accelerate the formation of advanced glycosylation end-products (AGEs) which in turn generate more free radicals (Giugliano et al., 1996; Vlassara, 1994). Antioxidant defences may also be impaired in diabetes, thereby contributing to net oxidative stress.

**Free radicals and diabetes**

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals (Halliwell and Gutteridge, 1999). There are other reactive molecules particularly derived from oxygen, which are not radicals (e.g. hydrogen peroxide (H$_2$O$_2$)). Singlet oxygen (O$_2^1$) is characterized by the anti-parallel spin of its two unpaired electrons, which is highly reactive compared to normal triplet oxygen. These uncoupled electrons are very reactive with adjacent molecules such as lipids, proteins and carbohydrates and can cause cellular damage (Kuhn, 2003).

The global term ROS includes oxygen-derived free radicals such as superoxide (O$_2^*$), hydroxyl (OH*), alkoxy (RO*) and peroxyl (ROO*) radicals plus non-radical derivatives of oxygen, namely, hydrogen peroxide (H$_2$O$_2$), singlet oxygen (O$_2^1$) and ozone (O$_3$) (Halliwell and Gutteridge, 1999). H$_2$O$_2$ is a product of a variety of cellular reactions such as amino acid oxidation, normal respiration and superoxide dismutation by superoxide dismutase. Although it is not a free radical and is more stable than free radicals, H$_2$O$_2$ possesses a serious threat to cells because it can react with O$_2$ or transition metals to form the serious hydroxyl radical (Robert, 1998).
Free radical production caused by hyperglycemia may occur via at least four different routes: i) increased glycolysis (Vaag et al., 1992); ii) intercellular activation of sorbitol (polyol) pathway (Williamson et al., 1993); iii) autooxidation of glucose (Wolff et al., 1991) and iv) non-enzymatic protein glycation (Ceriello et al., 1992).

Free radicals have been recognized as intermediates of some biological redox reactions essential for the maintenance of life. Biological materials, particularly membranes, contain high concentrations of unsaturated lipids. In the presence of a free radical initiator and oxygen they may be oxidized. This process is known as lipid peroxidation (PLA, 1976) and it has been implicated as a general biological degenerative reaction. It has been reported that glucose might be autooxidized generating free radicals (Curcio and Ceriello, 1992).

Glycated proteins produce free radicals and hydrogen peroxide in diabetes mellitus. The increased glycation of proteins may induce an increase in free radical production and affect several enzyme activities, and then accelerate atherogenesis as a result of oxidative modifications to the vascular membrane lipids.

**Lipid peroxidation and diabetes**

Lipid peroxidation is an oxidative deterioration of lipids containing a number of carbon-carbon double bonds. Among biological molecules lipids are the most susceptible to oxidative damage or peroxidation. Lipid hydroperoxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself. Their formation occurs in enzymic or non-enzymic reaction involving activated chemical species known as “reactive oxygen species” (ROS) (Fridorich, 1988), which are responsible for toxic effects in the body via various tissue damages. Increased lipid peroxidation is generally believed to be an important underlying cause of the initiation of oxidative stress related to various tissue injury and cell death, and further progression of many acute and chronic diseases (Halliwell and Gutteridge, 1999).
Lipid peroxidation is a radical mediated chain process involving 3 sequences: Initiation, propagation and termination. The process of lipid peroxidation is shown below (Figure 5). A free radical (R•) attacks fatty acid chain of membrane lipids (LH) by abstracting hydrogen atom thus leaving an unpaired electron. This is referred to as the initiation stage of lipid peroxidation (Gutteridge, 1995).

\[-\text{CH}_2- + \text{OH}^\bullet \rightarrow \text{CH}^\bullet + \text{H}_2\text{O}\]

Hydrogen atoms that are abstracted from a methylene group (-CH2-) have very high mobility. This attack easily generates free radicals from PUFA. The presence of a double bond in the fatty acids weakens the C-H bond on the carbon atom adjacent to the double bond and so makes hydrogen removal easier. Addition of molecular oxygen to alkyl radical (L•) leads to the formation of lipid peroxyl radical (LOO•), which in turn is capable of abstracting hydrogen from a second PUFA to form lipid hydroperoxide (LOOH). This is the propagation stage of LPO (Gupta and Kale, 1996).

The hydroperoxides react with transition metal ions (Fe^{2+}/Cu^{2+}) to form alkoxyl (LO•) and peroxyl radicals (LOO•) respectively. The peroxyl radical can form cyclic peroxides and cytotoxic aldehydes such as malondialdehyde (Halliwell and Gutteridge, 1986). L• can react with a lipid peroxyl radical (LOO•) to give non-initiating and non-propagating species such as the relatively stable dimers (LOOL). The chain reaction continues until the PUFA substrate is completely consumed. This is known as the termination stage.
Figure 5. The chemistry of oxygen radical induced membrane lipid peroxidation (Halliwell and Gutteridge, 1989).
Antioxidants

Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and/or activators of antioxidative defense enzyme systems to suppress the radical damages in biological systems (Yu et al., 2002, Prior et al., 2005). Antioxidants are agents which scavenge the free radicals and prevent the damage caused by reactive oxygen species (ROS) and by reactive nitrogen species (RNS).

Antioxidants are classified as preventive and chain breaking antioxidants (Halliwell and Gutteridge, 1989; Buettner, 1993). Preventive antioxidants are those that reduce the rate at which new chains are initiated. Most of the preventive antioxidants act either by reducing hydroperoxides to molecular products without the production of radicals (eg. glutathione peroxidase, catalase) or by sequestering and inactivating transition metal catalysts (eg. ceruloplasmin and transferrin). Antioxidants that can trap radicals directly, thereby shortening the chain length are classified as chain breaking antioxidants. This class includes superoxide dismutase (a superoxide trap), β-carotene (a peroxy and singlet oxygen trap) and vitamin E (a peroxy trap) (Halliwell and Gutteridge, 1995).

The evaluation of the antioxidant properties of specific chemical scavengers is of particular value for their potential use in preventing or limiting the damage induced by free radicals. Several methods are now used to measure the antioxidant activity of a biological material. The presence of the antioxidant leads to the disappearance of these radical chromogens, the two most widely used being the ABTS⁺⁺ and the DPPH radicals. DPPH is a free radical that is acquired directly without preparation while ABTS⁺⁺ must be generated by enzymatic or chemical reactions (Sindhu Mathew and Emilia Abraham, 2006).
Alterations of antioxidant status in diabetes

Free radicals are continually produced in the body as a result of normal metabolic processes and the interaction with environmental stimuli. Under physiological conditions, a wide range of antioxidant defenses protects the adverse effects of free radical production in vivo (Revnanen et al., 1998). Under normal conditions, potential toxic ROS generated by mitochondrial respiratory metabolism are efficiently neutralized by cellular antioxidant defense mechanisms. However, this balance can be easily broken resulting in cellular dysfunction. The balance between proxidant and antioxidant systems is very important in many disease processes including diabetes mellitus and is probably related to the complications associated with the disease (Ferreira et al., 1999). In addition to the increased generation of free radicals in diabetes, impaired generation of naturally occurring antioxidants also result in increased oxidative injury by failure of protective mechanisms (Bloomgarden, 1997).

There are several references to indicate that increased lipid peroxidation and decreased SOD, CAT and GPx activity in various organs like liver, kidney, heart, lymphoid organs, lens and blood vessels etc., during diabetes (Yadav et al., 1997). The non-enzymatic scavengers, vitamin E, the main intra-cellular antioxidant, protects tissue against unwanted and destructive oxidation. GSH plays a key role in the liver in detoxification reactions and in regulating the thiosulphide status of the cell (Chavan et al., 2005). Vitamin C, in addition to directly scavenging the free radicals in the cytoplasm, also participates in the regeneration of vitamin E and antioxidant peptide, glutathione (Bendich, 1997). The main principle of vitamin E is to break and terminate the free radical chain reaction in most tissues (Halliwell and Gutteridge, 1999).
Pathways leading to hyperglycemia-induced cell damage

The mechanisms by which diabetes can lead to hyperglycemia-induced oxidative stress are multifactorial (De Vriese et al., 2000) and include the following:

Advanced glycosylation end products (AGE)

ROS in diabetes may be the result of the non-enzymatic interaction of glucose with the amino side chains on proteins or lipoproteins (in particular lysine), through a series of oxidative and non-oxidative reactions, to the irreversible formation of Schiff-bases called advanced glycosylation end products (AGE), or Maillard products. AGE can accumulate in tissue over time and can either on their own or via their receptors found on endothelial cells, smooth muscle cells and macrophages inactivate enzymes by altering their structure and function, thus promoting ROS formation.

Polyol pathway

In tissue that does not require insulin for cellular glucose uptake such as blood vessels, hyperglycemia activates the polyol pathway in which aldose reductase reduces the aldehyde form of glucose to sorbitol. As this reaction requires NADPH, an increase in the polyol pathway flux may result in the depletion of cellular NADPH, which is a cofactor for many enzymes including NOS and the antioxidant glutathione reductase. In addition, as glucose is metabolized via the polyol pathway, it causes sorbitol to accumulate. Excessive amounts of sorbitol can lead to cellular osmotic stress (Obrosova et al., 2003) and this alters the antioxidant potential of the cell; and this increases ROS mediated damage of cellular proteins and lipids.

Activation of protein kinase C

Activation of PKC has now been demonstrated in all the vascular tissues involved in diabetic complications (Koya and King, 1998). Hyperglycemia causes
the de novo synthesis of diacylglycerol (DAG), the physiological activator of PKC, mainly through the glycolytic pathway. Although the ability of PKC to induce ROS is well established (Inoguchi et al., 2000; Cosentino et al., 2003), the mechanisms by which PKC induces vascular dysfunction remain unclear. Increased DAG content activates protein kinase C, and leads to several pathologies of diabetic complications (Brownlee, 2001). The activation of PKC can regulate many cellular functions including vascular permeability, contractility, cellular proliferation and signal transduction mechanisms. A common denominator for these abnormalities may be the formation of ROS.

**Experimental induction of diabetes**

**Diabetogenic Agents**

Many chemicals are used for the induction of diabetes mellitus in the animal models for testing new antihyperglycemic drugs. The most commonly used chemicals for the induction of diabetes in the experimental animals are alloxan and streptozotocin. The greatest disadvantage of alloxan diabetes model is multiorgan damage; hence this diabetogen is not widely employed to study the anti-diabetic effect of newer agents (Grussner et al., 1993).

**Streptozotocin (STZ)**

It is now well established that streptozotocin selectively destroys the pancreatic cells and produces hyperglycaemia (Gilman, 1990). Hence, streptozotocin is commonly employed for the induction of diabetes mellitus in experimental rats (Tomlinson et al., 1992).

Streptozotocin is produced by a strain of *Streptomyces achromogens*. STZ, a glucosamine-nitrosourea compound, has a chemical name of 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose (C$_8$H$_{15}$N$_3$O$_7$). The structure is composed of a nitrosourea moiety with a methyl group attached at one end and a glucose molecule at the other (Weiss, 1982).
Mode of action of streptozotocin

STZ is diabetogenic because it selectively destroys the insulin-producing beta cells by inducing necrosis. It is postulated that the selective beta-cell toxicity of STZ is related to the glucose moiety in its chemical structure, which enables STZ to enter the cell via the low affinity glucose transporter GLUT2 in the plasma membrane (Elsner, 2000). It is generally accepted that the cytotoxicity produced by STZ depends on DNA alkylation and subsequent activation of poly ADP-ribose synthetase causes rapid and lethal depletion of NAD in pancreatic β-islets, thereby causing cell death. Several lines of evidences indicate that free radicals, highly reactive carbonium radicals originating from the decay of STZ molecules might increase the production of oxygen free radicals including hydroxyl radicals and nitric oxide, may play an essential role in the mechanism of β-cell damage and diabetogenic effect of STZ (Halliwell and Gutteridge, 1999).
Management of Diabetes Mellitus

The treatment for Diabetes comprises include short-term and long-term goals, diet, lifestyle changes (exercise, smoking cessation), and medications (e.g., oral antidiabetic agents and insulin).

Diet and lifestyle changes

Medical nutrition therapy is an essential component of diabetes management; unfortunately, patient is adherence to nutrition principles is one of the most challenging aspects of diabetes care. A goal of medical nutrition therapy is to achieve and maintain blood glucose concentrations as close to normal as possible by balancing food intake with antidiabetic drug therapy and physical activity levels. Not more than 30% of the total daily caloric intake should come from fats;
10% to 20% from protein, and the balance of daily calories from carbohydrates. Exercise improves insulin sensitivity and glycaemic control, especially in patients with mild diabetes or a high degree of insulin resistance (ADA, 2000).

**Insulin therapy**

Insulin is an important hormone needed by the human body to utilize carbohydrates, protein, and fats. However, in type 1 diabetes the pancreas does not produce insulin, and replacement therapy is required with exogenous insulin. Type 2 diabetics, on the other hand, have a problem with either the secretion of insulin or have become insulin-resistant; thus, the common name for the condition is non insulin dependent diabetes mellitus. Insulin injections have thus become compulsory daily component of therapy for type 1 diabetics. Insulin injections however, are not always necessary for the treatment and control of diabetes in type 2 diabetics (Buse, 1999).

**Oral hypoglycemic agents**

The oral antidiabetic agents that are used in the treatment of type 2 diabetes fall into four categories: beta cell stimulators (sulfonylureas, Meglitinide), biguanides (Metformin), α-glucosidase inhibitors, and thiazolidinediones (Vaaler, 2000). The beta cell stimulators act at the level of the pancreatic beta cells to stimulate insulin release. They require the presence of functioning beta cells, and are used only in the treatment of type 2 diabetes, and have the potential for producing hypoglycemia. The sulfonylureas reduce blood glucose by stimulating the release of insulin from the beta cells in the pancreas and increasing the sensitivity of peripheral tissues to insulin. Repaglinide and nateglinide are nonsulfonylurea beta cell stimulators. These agents, which are rapidly absorbed from the gastrointestinal tract, are taken shortly before meals. Both repaglinide and nateglinide can produce hypoglycemia; thus, proper timing of meals in relation to drug administration is important.
Metformin, the only currently available biguanide, inhibits hepatic glucose production and increases the sensitivity of peripheral tissues to the actions of insulin. Secondary benefits of metformin therapy include weight loss and improved lipid profiles. Unlike the sulfonylureas, whose primary action is to increase insulin secretion, metformin exerts its beneficial effects on glycemic control through decreased hepatic glucose production (main effect) and increased peripheral use of glucose. This medication does not stimulate insulin secretion; therefore, it does not produce hypoglycemia. Because of the risk of lactic acidosis, metformin is contraindicated in the people with elevated serum creatinine levels, clinical and laboratory evidence of liver disease, or with conditions associated with hypoxemia or dehydration.

The α-glucosidase inhibitors block the action of the brush border enzymes in the small intestine that break down complex carbohydrates. By delaying the breakdown of complex carbohydrates, the α-glucosidase inhibitors delay the absorption of carbohydrates from the gut and blunt the postprandial increase in plasma glucose and insulin levels. The postprandial hyperglycemia probably accounts for sustained increases in HbA1c levels.

The thiazolidinediones (TZDs), or glitazones, are the only class of drugs that directly target insulin resistance, a fundamental defect in the pathophysiology of type 2 diabetes. The TZDs improve glycemic control by increasing insulin sensitivity in the insulin-responsive tissues of liver, skeletal muscle, and fat allowing the tissues to respond to endogenous insulin more efficiently without increased output from already dysfunctional beta cells. A secondary effect is the suppression of hepatic glucose production. The mechanism of action of the TZDs is complex and not fully understood but is believed to be associated with binding of the drug to a nuclear receptor that plays a role in the regulation of genes
involved in lipid and glucose metabolism (Schoonjans and Auwerx, 2000). Because of a potential problem with liver toxicity, liver enzymes should be measured when these drugs are used.

**Ethnomedical importance of natural remedies**

Diabetes mellitus is becoming pandemic and despite the recent upsurge in the advent of new drugs to treat and prevent the condition, its prevalence continues to increase. Despite the great strides that have been made in the understanding and management of diabetes, the disease and disease related complications are steadily on the increase. Currently available drug regimes for management of diabetes mellitus have certain drawbacks and therefore, there is a need for safer and more effective antidiabetic drugs. Many oral hypoglycaemic agents, such as biguanides and sulfonylureas are available along with insulin for the treatment of diabetes mellitus (Holman and Turner, 1991), but these synthetic agents can produce serious side effects, and in addition, they are not suitable for use during pregnancy (Larner, 1985 and Valiathan, 1998). In addition, the cost of administering modern antidiabetic drugs is beyond the reach of people in the low income group and those living in the rural areas. Therefore, search for safe and more effective and cost effective agents has continued to be an important area of active research. This necessitates identifying indigenous natural resources in order to develop them as new therapeutics (Li *et al.*, 2004). The belief that natural medicines are much safer than synthetic drugs has gained popularity in recent years and this has lead to tremendous growth of phytopharmaceutical usage (Bhattaram *et al.*, 2002).

One of the problems encountered in crude plant drugs is the batch to batch variation in their efficacies. Such variations could arise due to natural genetic variation, seasonal variation, differences in the soil and climatic conditions, nutritional status, etc. of the medicinal plants. So, phytochemical approach of plant
drug discovery emphasizes the development of pure phytochemicals as drugs. Later this shifted treatment of diabetes towards the usage of synthetic oral hypoglycaemic agents. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigations on hypoglycaemic agents from medicinal plants have become more important (WHO, 1980)

Figure 8. Pharmacological treatment of hyperglycaemia according to site of action (Stumvoll et al., 2005)
**Emblica officinalis** Gaertn.

*Emblica officinalis* Gaertn. (*Phyllanthus emblica* L.; *Dichelactina nodicaulis* Hance; *Emblica arborea* Raf.; *Phyllanthus glomeratus* Wall.; *Cicca emblica* Kurz.; *Diasperus emblica* Kuntze) is a medium to large deciduous tree belonging to a small subgenus of trees belonging to the family Euphorbiaceae growing in India, Sri Lanka, Pakistan, Uzbekistan, S.E. Asia, and China. *Emblica* (Amla in Hindi) grows wild and is also cultivated up to a height of 1400m a.s.l.; in India, the most common cultivars are “Chakaiya”, “Banarsi”, and “Francis” (Scartezzini and Speroni, 2000). The previous name, *Phyllanthus emblica* L., was attributed by Linneaus with reference to a peculiar characteristic of this plant. The branches of this tree are oddly flattened in the manner of a leaf; the flowers bloom from the edges of these leaf-like branches, thus the name *Phyllanthus*, from the Greek words “phyllon” (leaf) and “anth`os” (flower). The name *Emblica* derives almost certainly from the “corruption” of the Sanskrit name “Amlika”, although some authors believe that it could derive from the “corruption” of the Arabic word “Embelgi” used by Arabic physicians to name its fruit. In Sanskrit, *Emblica* has many synonyms: Amalaki (pure, clean), Dhatriphala (nurse fruit), Amritaphala (fruit of immortality), and others. All of these synonyms show how important this plant is in traditional Indian medicine. In Malaysia, this plant is so renowned that a city and a river bear its name: Malacca. The fruits of *Emblica* are widely consumed raw, cooked, or pickled, but they are also the principal constituents of many Ayurvedic preparations (Scartezzini and Speroni, 2000). Indeed, *Emblica* is one of the most important plants of Ayurved, the Indian Traditional Medicine. According to the two main classic texts on Ayurved, *Charak Samhita* and *Sushrut Samhita*, Amalaki is regarded as “the best among rejuvenative herbs”, “useful in relieving cough and skin disease”, and “the best among the sour fruits”. There are two historically ascertained events in which this
plant was used for medical purposes: the famine of 1939-1940 and the cases of scurvy in the Indian army in Nassirdab, today known as Rajasthan, in 1837 (Srinivasan, 1944).

In recent years, new pharmacological activities have been found for *Emblica*: it has cytoprotective activity against chromium (Sai Ram *et al.*, 2003), protects from oxidative stress in ischemic-reperfusion injury (Rajak *et al.*, 2004), shows antivenom capacity (Alam and Gomes, 2003), ameliorates hyperthyroidism and hepatic lipid peroxidation (Panda and Kar, 2003), displays antiproliferative activity on MCF7 and MDA-MB-231 breast cancer cell lines (Lambertini *et al.*, 2003), shows antitussive activity (Nosal’ova *et al.*, 2003), and induces apoptosis in Dalton’s Lymphoma Ascites and CeHa cell lines (Rajeshkumar *et al.*, 2003).

**Emblicanin**

Emblicanin is a type of antioxidant found in Amla aka the Indian gooseberry. Amla is known as *Emblica officinalis* in botanical terms. Emblicanin is different from most other antioxidants as it is a pro-oxidation free cascading antioxidant.

![Figure 9: Structure of Emblicanin A](image-url)
Many antioxidants intrinsically have a pro-oxidant action, especially in the presence of transition metals like iron and copper. Through a series of reactions with oxygen species known as Fenton reaction, iron causes generation of highly toxic hydroxyl radical with subsequent damage to biomolecules. It means that the antioxidants which are meant to scavenge free radicals themselves create free radicals.

While most antioxidants go directly from an active to an inactive role, Emblicanin utilizes a multilevel cascade of antioxidant compounds resulting in prolongation of its antioxidant capabilities.

Emblicanin A (one of the key compounds in Emblicanin) aggressively seeks and attacks free radicals. After it neutralizes a free radical, Emblicanin A is transformed into Emblicanin B, another antioxidant. Emblicanin B in turn also attacks the free radicals and is transformed into Emblicanin oligomers. This makes emblicanin one of the best free radical scavenging antioxidant.