INTRODUCTION

Diabetes mellitus (DM) or simply diabetes is 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 different 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, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia (American Diabetic Association, Diabetes Care, 2010).

The term “Diabetes mellitus” is derived from the Greek words dia (=through), bainein (=to go) and diabetes literally means pass through. The disease causes loss of weight as if the body mass is passed through the urine. Although it was known for centuries that the urine of patients with diabetes was sweet, it was not until 1674 that physician named Willis coined the term Diabetes Mellitus (from the Greek word for honey) (Vasudevan et al., 2005). It was only in 1776 that Dobson (Britain) firstly confirmed the presence of excess sugar in urine and blood as a cause of their sweetness (Ahmed et al., 2002). Diabetes mellitus was first identified as a disease associated with "sweet urine," and excessive muscle loss in the ancient world. Elevated levels of blood glucose (hyperglycemia) lead to spillage of glucose into the urine, hence the term sweet urine.

In DM body fails to produce enough insulin and hence excess glucose accumulates in the blood instead of being utilized or stored. This situation is so called “starvation in the midst of plenty”. The body responds as if it was in the fasting state with stimulation of glycogenolysis, Gluconeogenesis and lipolysis. The glucose absorbed during a meal is not metabolized at the normal rate and therefore accumulates in the blood (hyperglycemia) to be excreted in the urine.
(glycosurea). Glucose in the urine causes osmotic diuresis, leading to increase in the urine production (polyurea). Stimulation of protein breakdown to provide amino acids for Gluconeogenesis results in muscle wasting and weight loss. Hyperglycemia, glycosurea, polyurea, polydypsia, polyphagia and unexpected weight loss are the classical symptoms of DM (American Diabetic Association, Diabetes Care., 2012).

CLASSIFICATION AND DIAGNOSIS OF DIABETES MELLITUS

Classification

According to the recommendations of American Diabetes Association (ADA) and the world health organization (WHO), the new classification system (Table 1) identifies four clinical classes of diabetes mellitus (DM) (American Diabetic Association, Diabetes Care, 2010).

The vast majority of diabetic patients are classified into one of two broad categories: Type 1 diabetes, which is caused by an absolute deficiency of insulin, and type2 diabetes, which is characterized by the presence of insulin resistance with an inadequate compensatory increase in insulin secretion. In addition, women who develop diabetes during their pregnancy are classified as having gestational diabetes. Finally, there are a variety of uncommon and diverse types of diabetes which are caused by infections, drugs, endocrinopathies, pancreatic destruction, and genetic defects. These unrelated forms of diabetes are classified separately.

1. Type 1 Diabetes Mellitus (T1DM) (Insulin Dependent Diabetes Mellitus, IDDM):

Type 1 DM is caused by absolute insulin deficiency due to destruction of pancreatic β-cells principally via an autoimmune reaction that can be triggered by different factors (Chhabra et al., 2013). It can also develop in association with certain hereditary factors, such as Human Leukocyte Antigen (HLA) alleles. Typically, destruction of pancreatic β-cells progresses to absolute deficiency in insulin. This condition develops rapidly in young people and has been found to occur in any age group (Chhabra et al., 2013). Similarly, auto antibodies against islet antigens (islet associated antibodies) have been shown to increase in the early phase of the disease. Hence, pancreatic β-cell destruction involves autoimmune mechanisms. Therefore, type 1 diabetes mellitus is also known as ‘autoimmune’ type 1 diabetes mellitus (Chhabra et al., 2013; Ting et al., 2012; Thompson et al., 2012).
Type 1 DM is usually characterized by the presence of anti-glutamic acid decarboxylase (anti-GAD), islet cell or insulin antibodies which identify the autoimmune processes that lead to beta-cell destruction. Sequence of molecular events leading to Type 1 diabetes is shown in Figure 1. Type 1 DM is of two types like immune mediated diabetes and idiopathic diabetes.

Figure 1: Sequence of molecular events leading to Type 1 diabetes.
(Source: Kharagjitsing, 2012).

Immune-mediated diabetes

This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes, type I diabetes, or juvenile- onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas. Markers of the immune destruction of the β-cell include islet cell auto antibodies, auto antibodies to insulin, auto antibodies to glutamic acid decarboxylase (GAD65), and auto antibodies to the tyrosine phosphatases IA-2 and IA-2β. The disease has strong HLA associations, with linkage to the DQA and DQB genes, and it is influenced by the DRB genes. These HLA-DR/DQ alleles can be either predisposing or protective. In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others.
(mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or keto acidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual β-cell function sufficient to prevent keto acidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for keto acidosis. At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide. Immune mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in the 8th and 9th decades of life. These patients are also prone to other autoimmune disorders such as Graves’s disease, Hashimoto’s thyroiditis, Addison’s disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia (American Diabetic Association, Diabetes Care, 2010).

**Idiopathic diabetes**

Some forms of Type 1 DM have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of patients with type 1 diabetes falls into this category, most are of African or Asian ancestry. This form of diabetes is strongly inherited, lacks immunological evidence for β-cell autoimmunity, and is not HLA associated (American Diabetic Association, Diabetes Care, 2010).

2. **Type 2 Diabetes Mellitus (T2DM) (Non Insulin Dependent Diabetes Mellitus, NIDDM):**

This form of diabetes, which accounts for ~90–95% of those with diabetes, previously referred to as non insulin-dependent diabetes mellitus, Type 2 diabetes, or adult-onset diabetes mellitus, encompasses individuals who have the insulin resistance and usually have relative (rather than absolute) insulin deficiency atleast initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. 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.
3. OTHER SPECIFIC TYPES OF DIABETES

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. They include, for example, fibrocalculous pancreatitis, a form of diabetes which was formerly classified as one type of malnutrition-related diabetes mellitus.

Genetic defects of the β-cell

Several forms of diabetes are associated with monogenic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 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. They are inherited in an autosomal dominant pattern. Abnormalities at six genetic loci on different chromosomes have been identified to date. The most common form is associated with mutations on chromosome 12 in a hepatic transcription factor referred to as hepatocyte nuclear factor (HNF)-1α. A second form is associated with mutations in the glucokinase gene on chromosome 7p and results in a defective glucokinase molecule. The less common forms result from mutations in other transcription factors, including HNF-4α, HNF-1β, insulin promoter factor (IPF)-1, and NeuroD1.

Point mutations in mitochondrial DNA have been found to be associated with diabetes mellitus and deafness. The most common mutation occurs at position 3243 in the tRNA of leucine gene, leading to an A-to-G transition. An identical lesion occurs in the MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like syndrome) however, diabetes is not part of this syndrome, suggesting different phenotypic expressions of this genetic lesion.

Genetic abnormalities that result in the inability to convert proinsulin to insulin have been identified in a few families, and such traits are inherited in an autosomal dominant pattern. The resultant glucose intolerance is mild. Similarly, the production of mutant insulin molecules with resultant impaired receptor binding has also been identified in a few families and is associated with an autosomal inheritance and only mildly impaired or even normal glucose metabolism (American Diabetic Association, Diabetes Care, 2010).
Genetic defects in insulin action

Mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. Women may be virilised and have enlarged, cystic ovaries. In the past, this syndrome was termed type A insulin resistance. Leprechaunism and the Rabson-Mendenhall syndrome are two pediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance. The former has characteristic facial features and is usually fatal in infancy, while the latter is associated with abnormalities of teeth and nails and pineal gland hyperplasia (American Diabetic Association, Diabetes Care, 2010).

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. Cystic fibrosis and hemochromatosis will also damage β-cells and impair insulin secretion. Fibrocalculous pancreatopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcifications identified on X-ray examination. Pancreatic fibrosis and calcium stones in the exocrine ducts have been found at autopsy (American Diabetic Association, Diabetes Care, 2010).

Endocrinopathies

Several hormones (e.g., growth hormone, cortisol, glucagon, and epinephrine) antagonize insulin’s action. Excess amounts of these hormones (e.g., acromegaly, Cushing’s syndrome, glucagonoma, pheochromocytoma, respectively) can cause diabetes. Somatostatinoma - and aldosteronoma - induced hypokalemia can cause diabetes, at least in part, by inhibiting insulin secretion (American Diabetic Association, Diabetes Care, 2010).

Drug- or chemical-induced diabetes

Many drugs can impair insulin secretion. These drugs may not, by themselves, cause diabetes but they may precipitate diabetes in persons with insulin resistance. In such cases, the classification is ambiguous, as the primacy of beta-cell dysfunction or insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β-cells. Such drug reactions fortunately are rare. There are also
many drugs and hormones that can impair insulin action. Examples include nicotinic acid and glucocorticoids. Patients receiving \( \alpha \)-interferon have been reported to develop diabetes associated with islet cell antibodies and, in certain instances, severe insulin deficiency (American Diabetic Association, Diabetes Care, 2010).

**Infections**

Certain viruses have been associated with \( \beta \)-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.

**Uncommon forms of immune-mediated diabetes**

In this category, there are two known conditions, and others are likely to occur. The stiff-man syndrome is an autoimmune disorder of the central nervous system characterized by stiffness of the axial muscles with painful spasms. Patients usually have high titers of the GAD auto antibodies, and approximately one-third will develop diabetes.

Anti–insulin receptor antibodies can cause diabetes by binding to the insulin receptor, thereby blocking the binding of insulin to its receptor in target tissues. However, in some cases, these antibodies can act as an insulin agonist after binding to the receptor and can thereby cause hypoglycemia. Anti–insulin receptor antibodies are occasionally found in patients with systemic lupus erythematosus and other autoimmune diseases. As in other states of extreme insulin resistance, patients with anti–insulin receptor antibodies often have Acanthosis nigricans. In the past, this syndrome was termed type B insulin resistance.

**Other genetic syndromes sometimes 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 \( \beta \)-cells at autopsy. Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy, and neural deafness (American Diabetic Association, Diabetes Care, 2010).
4. GESTATIONAL DIABETES MELLITUS

GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Although most cases resolve with delivery, the definition applies regardless of whether insulin or only diet modification is used for treatment or whether the condition persisted after pregnancy and did not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy. As the ongoing epidemic of obesity and diabetes has led to more Type 2 diabetes in women of child bearing age, the number of pregnant women with undiagnosed Type 2 diabetes has increased.

After deliberations in 2008–2009, the International Association of Diabetes and Pregnancy Study Groups (IADPSG), an international consensus group with representatives from multiple obstetrical and diabetes organizations, including the American Diabetes Association (ADA), recommended that high-risk women found to have diabetes at their initial prenatal visit, using standard criteria, receive a diagnosis of overt, not gestational, diabetes. Approximately 7% of all pregnancies (ranging from 1 to 14%, depending on the population studied and the diagnostic tests employed) are complicated by GDM, resulting in more than 200,000 cases annually. GDM represents nearly 90% of all pregnancies complicated by diabetes. Deterioration of glucose tolerance occurs normally during pregnancy, particularly in the 3rd trimester. During pregnancy, increasing blood glucose levels increase the risk for both mother and fetus and require treatment to reduce problems for the mother and infant. Treatment may include diet, regular physical activity, or insulin. Shortly after pregnancy, 5% to 10% of women with gestational diabetes continue to have high blood glucose levels and are diagnosed as having diabetes, usually Type 2. Women at high risk are those older than 25 years of age with positive family history of diabetes mellitus and obesity. The increasing demand of insulin during pregnancy and hormonal changes are predisposing these women to the development of gestational diabetes mellitus (Mealey et al., 2007).

The risk factors for gestational diabetes are similar to those for Type 2 diabetes. The occurrence of gestational diabetes itself is a risk factor for developing recurrent gestational diabetes with future pregnancies and subsequent development of Type 2 diabetes. Also, the children of women who had gestational diabetes during pregnancies may be at risk of developing obesity and diabetes.
Diagnosis of diabetes

Multiple laboratory tests are used in the diagnosis and management of patients with diabetes mellitus. Measurement of plasma glucose remains the sole diagnostic criterion for diabetes. Monitoring of glycemic control is performed by the patients, who measure their own plasma or blood glucose with meters, and by laboratory analysis of glycated hemoglobin. The potential roles of noninvasive glucose monitoring, genetic testing, auto antibodies, microalbumin, proinsulin, C-peptide, and other analytes are addressed (The American Association for Clinical Chemistry., 2002). For decades, the diagnosis of diabetes was based on plasma glucose criteria, either the “fasting plasma glucose” (FPG) or the 2-h value in the 75-g oral glucose tolerance test (OGTT) (American Diabetes Association., 2010).

According to expert committee appointed by ADA in 1997, the criteria for diagnosing the Diabetes include,

- FPG <100 mg/dL (5.6 mmol/L) = normal fasting glucose
- FPG 100–125 mg/dL (5.6–6.9 mmol/L) = IFG (impaired fasting glucose)
- FPG ≥126 mg/dL (7.0 mmol/L) = provisional diagnosis of diabetes

The corresponding categories when the OGTT is used are the following:

- 2-h post load glucose <140 mg/dL (7.8 mmol/L) = normal glucose tolerance
- 2-h post load glucose 140–199 mg/dL (7.8 –11.1 mmol/L) = IGT (impaired glucose tolerance)
- 2-h post load glucose ≥200 mg/dL (11.1 mmol/L) = provisional diagnosis of diabetes.

Patients with IFG and/or IGT are now referred to as having “pre-diabetes” indicating the relatively high risk for development of diabetes in these patients. IFG and IGT are associated with the metabolic syndrome, which includes obesity (especially abdominal or visceral obesity), dyslipidemia of the high-triglyceride and/or low-HDL type, and hypertension. Note that many individuals with IGT are euglycemic in their daily lives. Individuals with IFG or IGT may have normal or near normal glycated hemoglobin levels. Individuals with IGT often manifest hyperglycemia only when challenged with the oral glucose load used in the standardized OGTT.
New guidelines for the diagnosis of diabetes mellitus

In 2009, an International Expert Committee that included representatives of the ADA, the International Diabetes Federation (IDF), and the European Association for the Study of Diabetes (EASD) recommended the use of the HbA1c test to diagnose diabetes, with a threshold of \( \geq 6.5\% \) (International Expert Committee., 2009) (Table 1), and ADA adopted this criterion in 2010 (American Diabetes Association., 2010).

Table 1: Diagnosis of diabetes (International Expert Committee, 2009).

<table>
<thead>
<tr>
<th>S.NO</th>
<th>New Criteria for the diagnosis of diabetes</th>
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<tbody>
<tr>
<td>1.</td>
<td>HbA1c ( \geq 6.5% ). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.* or</td>
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<tr>
<td>2.</td>
<td>FPG ( \geq 126 \text{ mg/dL} ) (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.* or</td>
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<tr>
<td>3.</td>
<td>2-h plasma glucose ( \geq 200 \text{ mg/dL} ) (11.1 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 g anhydrous glucose dissolved in water.* or</td>
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<tr>
<td>4.</td>
<td>In a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose ( \geq 200 \text{ mg/dL} ) (11.1 mmol/L).</td>
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</table>

*In the absence of unequivocal hyperglycemia, result should be confirmed by repeat Testing.

PREVALENCE OF DIABETES

The prevalence of diabetes is rising all over the world due to population growth, aging, urbanization and an increase of obesity and physical inactivity. Unlike in the West, where older persons are most affected, diabetes in Asian countries is disproportionately high in young to middle-aged adults. This could have long-lasting adverse effects on a nation’s health and economy, especially for developing countries. The International Diabetes Federation (IDF) estimates the total number of people in India with diabetes to be around 50.8 million in 2010, rising to 87.0 million by 2030.
According to recent estimates, approximately 285 million people worldwide (6.6%) in the 20–79 year age group will have diabetes in 2010 and by 2030, 438 million people (7.8%) of the adult population, is expected to have diabetes (IDF Diabetes Atlas, 2009). The largest increases will take place in the regions dominated by developing economies. The global increase in the prevalence of diabetes is due to population growth, aging, urbanization and an increase of obesity and physical inactivity. Estimated global healthcare expenditures to treat and prevent diabetes and its complications are expected to total at least 376 billion U.S. Dollars (USD) in 2010. By 2030, this number is projected to exceed some USD 490 billion (IDF Diabetes Atlas, 2009).

**The Global Burden**

- 366 million people have diabetes in 2011; by 2030 this will have risen to 552 million
- The number of people with Type 2 diabetes is increasing in every country
- 80% of people with diabetes live in low- and middle-income countries
- The greatest number of people with diabetes are between 40 to 59 years of age
- 183 million people (50%) with diabetes are undiagnosed
- Diabetes caused 4.6 million deaths in 2011
- Diabetes caused at least USD 465 billion dollars in healthcare expenditures in 2011;
- 11% of total healthcare expenditures in adults (20-79 years)
- 78,000 children develop Type 1 diabetes every year.

Diabetes prevalence is higher in men than in women, but there are more women with diabetes than men. In developing counties, the urban population with diabetes is projected to double between 2000 and 2030 (Figure 2). Diabetes is a global problem with devastating human, social and economic impact. The International Diabetes Federation (IDF) currently states that the top 5 countries with the highest number of diabetic patients are as follows, (Table 2).

- China
- India
- United States of America
- Russia
- Brazil
It is estimated that the total number of people with diabetes in 2010 to be around 50.8 million in India, rising to 87.0 million by 2030 (IDF Diabetes Atlas, 2009). According to the World Health Organization (WHO) criteria, the prevalence of known diabetes was 5.6% and 2.7% among urban and rural areas, respectively (Mohan et al., 2009). Diabetes showed positive and independent associations with age, body mass index (BMI), waist-to-hip ratio, a family history of diabetes, monthly income and sedentary physical activity. Age, BMI and a family history of diabetes showed associations with IGT. The current estimate for 2010 of 285 million adults with diabetes is 67% higher than the 2004 published estimate for the year 2000 (Wild et
al., 2004), and their 2030 estimate of 439 million is 20% higher than the same studies estimate for 2030 (Wild et al., 2004).

Table 2: Showing the top 10 countries/territories of number of people with diabetes (20-79 years), 2011 and 2030. (Source: IDF Diabetes Atlas, 2011).

<table>
<thead>
<tr>
<th>S.NO</th>
<th>COUNTRY/TERRITORY</th>
<th>2011 MILLIONS</th>
<th>S.NO</th>
<th>COUNTRY/TERRITORY</th>
<th>2030 MILLIONS</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>China</td>
<td>90</td>
<td>1</td>
<td>China</td>
<td>129.7</td>
</tr>
<tr>
<td>2</td>
<td>India</td>
<td>61.3</td>
<td>2</td>
<td>India</td>
<td>101.2</td>
</tr>
<tr>
<td>3</td>
<td>United States of America</td>
<td>23.7</td>
<td>3</td>
<td>United States of America</td>
<td>29.6</td>
</tr>
<tr>
<td>4</td>
<td>Russian Federation</td>
<td>12.6</td>
<td>4</td>
<td>Brazil</td>
<td>19.6</td>
</tr>
<tr>
<td>5</td>
<td>Brazil</td>
<td>12.4</td>
<td>5</td>
<td>Bangladesh</td>
<td>16.8</td>
</tr>
<tr>
<td>6</td>
<td>Japan</td>
<td>10.7</td>
<td>6</td>
<td>Mexico</td>
<td>16.4</td>
</tr>
<tr>
<td>7</td>
<td>Mexico</td>
<td>10.3</td>
<td>7</td>
<td>Russian Federation</td>
<td>14.1</td>
</tr>
<tr>
<td>8</td>
<td>Bangladesh</td>
<td>8.4</td>
<td>8</td>
<td>Egypt</td>
<td>12.4</td>
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<tr>
<td>9</td>
<td>Egypt</td>
<td>7.3</td>
<td>9</td>
<td>Indonesia</td>
<td>11.8</td>
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<tr>
<td>10</td>
<td>Indonesia</td>
<td>7.3</td>
<td>10</td>
<td>Pakistan</td>
<td>11.4</td>
</tr>
</tbody>
</table>

China tops the list with 90.0 million people affected by diabetes, followed by India. This has 61.3 million affected people. The numbers are estimated to rise to 129.7 million and 101.2 million, respectively, by 2030. These estimates are likely to be under estimations as the prevalence data are mostly available for urban areas and reports from rural areas are scanty. With the rapid socioeconomic changes occurring in the rural areas, the prevalence of diabetes and other non communicable diseases (NCDs) are bound to increase several-fold. These diseases contribute largely to early morbidity and mortality among the population.
PREVALENCE IN INDIA

According to the International Diabetes Federation, 61.3 million people in India had diabetes in 2011. That Figure is projected to rise to 101.2 million by 2030 (IDF Diabetes Atlas, 5th ed., 2011). IDF data reveal that India has more diabetes than the United States. In fact, India is ranked second in the world in diabetes prevalence, just behind China. Diabetes has emerged as a major healthcare problem in India. According to the World Health Organization estimates, India had 32 million diabetic subjects in the year 2000 and this number would increase to 80 million by the year 2030 (Wild et al., 2004). The International Diabetes Federation (IDF) also reported that the total number of diabetic subjects in India is 41 million in 2006 and that this would rise to 70 million by the year 2025 (Sicree et al., 2006).

The Risk Factors for Diabetes in Indians are:

Age: Indians develop diabetes at a very young age, at least 10 to 15 years earlier than the western population. An early occurrence of diabetes gives ample time for development of the chronic complications of diabetes. The incidence of diabetes increases with age. In India, the life span has increased; hence more number of people with diabetes is being detected.

Family History: The prevalence of diabetes increases with a family history of diabetes. The risk of a child developing diabetes with a parental history increases above 50 per cent. A high incidence of diabetes is seen among the first degree relatives. Indians have a high genetic risk for diabetes as observed in Asian Indians who have migrated to other countries. They have been found to have a higher rate of diabetes as compared to the local population.

Central Obesity: The association of obesity with Type 2 Diabetes is well known. Even with an acceptable body weight range, weight gain could increase the risk of diabetes. An excess of body fat specially concentrated within the abdomen has an increased risk of diabetes. The cut-off limits for waist circumference for Indians have been recommended to be 90 cm for males and 80 cm for females. Abdominal obesity is defined by waist circumference above these limits.

Physical Inactivity and Sedentary Living: There is enough evidence to demonstrate that physical inactivity as an independent factor for the development of Type 2 diabetes. The availability of motorized transport and a shift in occupations combined with the plethora of television programmes has reduced the physical activity in all groups of populations.
Insulin Resistance: Asian Indians have been found to be more insulin resistant as compared to the white population. They have a higher level of insulin to achieve the same the blood glucose control. A cluster of factors consisting of abnormal fats (Dyslipidemia), high blood pressure, obesity, and abnormal glucose levels known as metabolic syndrome is highly prevalent in Asian Indians.

Urbanization: The developing countries like India are undergoing rapid urbanization. Urbanization is associated with increasing obesity, decreasing physical activity due to changes in lifestyle, diet and a change from manual work to less physical occupations.

Stress: The impact of stress both physical and mental along with lifestyle changes has a strong effect of increasing incidence of Type 2 Diabetes amongst persons with a strong genetic background.

EXPERIMENTAL DIABETIC ANIMAL MODEL

Diabetes can be induced by pharmacologic, surgical or genetic manipulations in several animal species. Most experiments in diabetes are carried out on rodents, although some studies are still performed in larger animals. The majority of studies published in the field of ethnopharmacology between 1996 and 2006 employed pharmacological models. Streptozotocin (STZ) and alloxan are by far the most frequently used diabetogenic drugs and this model has been useful for the study of multiple aspects of the disease.

The cytotoxic action of these diabetogenic agents is mediated by reactive oxygen species, but both drugs differ in their mechanism of action (Federiuk et al., 2004; Lei et al., 2005). Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, which causes rapid destruction of pancreatic β-cells (Szudelski, 2001). Streptozotocin enters the pancreatic β-cell via a glucose transporter-GLUT2 and causes alkylation of deoxyribonucleic acid (DNA). Furthermore, STZ induces activation of poly adenosine diphosphate ribosylation and nitric oxide release. As a result of STZ action, pancreatic β-cells are destroyed by necrosis (Mythili et al., 2004) (Figure. 4).
Figure 3: The chemical structure of GlcNAc and STZ

\[ \text{N-Acetylglucosamine} \quad \text{Streptozotocin} \]

Figure 4: Proposed mechanism of Streptozotocin-induced β-cell injury (Modified from Okamoto et al., 1985)
For several decades, the β-cell-specific toxin Streptozotocin (STZ), an analogue of GlcNAc (Figure 3), has been used to create animal models of diabetes, despite an incomplete understanding of how STZ actually causes β-cell death (Herr et al., 1967). The ability of STZ to act as a NO donor has led many investigators to postulate that NO is involved (Kroncke et al., 1995), but the diabetogenic effect of STZ in i.o cannot be readily duplicated with N-methyl-N-nitrosourea (MNU, the portion of STZ that actually donates NO) (Voss et al., 1988). Recently, STZ has been shown to inhibit the enzyme O-GlcNAc-selective N-acetyl-β-D-glucosaminidase (OGlcNAcase), which removes O-GlcNAc from protein, and is thus the final enzyme in the pathway of O-glycosylation in the β-cell (Liu et al., 2000).

Pancreatic β-cells have been proposed to be selectively sensitive to STZ because the enzyme responsible for transferring O-GlcNAc to proteins, OGlcnAc transferase (OGT) (Kreppel et al., 1997), is expressed at higher levels in the β-cell than in any other cell (Liu et al., 2000; Hanover et al., 1999). The potential problem with STZ is that its toxic effects are not restricted to pancreatic β-cells since it may cause renal injury (Valentovic et al., 2006), oxidative stress inflammation and endothelial dysfunction (Lei et al., 2005). Vacor, dithizone (diphenylthio-carbazone), and 8-hydroxy quinolone may also cause experimental diabetes, but their use in research is restricted due to their level of toxicity (Clark et al., 1994).

COMPLICATIONS OF DIABETES MELLITUS

Diabetes mellitus is associated with serious complications that can impair quality of life and function and lead to premature death. The complications of diabetes mellitus can be divided into 2 major types.

1. Acute metabolic complications
2. Chronic or long-term vascular complications

ACUTE COMPLICATIONS OF DIABETES MELLITUS

The acute metabolic complications of diabetes consist of diabetic ketoacidosis (DKA), hyper osmolar non-ketotic coma (HNC), lactic acidosis (LA), and hypoglycemia. DKA and HNC are related to insulin deficiency. Hypoglycemia results from the treatment of diabetes, either with oral agents or insulin. Although hypoglycemia may occur in conjunction with oral hypoglycemic therapy, it is more common in patients treated with insulin. LA is usually associated with other factors that may be related to diabetes, such as cardiovascular disease (acute myocardial
infarction) associated with hypoxia and excess lactic acid production. Pathogenesis of diabetic ketoacidosis and hyperglycemic hyperosmolar state are represented in Figure 5.

Figure 5: Pathogenesis of diabetic ketoacidosis (DKA) and Hyperglycemic Hyperosmolar State (HHS) (Source: English and Williams. 2004).

DKA is one of the major acute diabetic complications. DKA is clinically defined by absolute insulin deficiency with hyperglycemia (glucose levels usually >200 mg/dL) with increased lipolysis, increased ketone production, hyperketonemia (ketone levels positive at 1:4 dilution of serum or greater or beta hydroxybutyrate >0.5 mmol/L), and acidosis (pH ≤7.3 or bicarbonate ≤15 mEq/L). HNC is clinically defined by the presence of relative insulin deficiency and hyperglycemia, usually >1,000 mg/dL with associated elevated serum osmolality (>300 mosm/kg), dehydration, and stupor, progressing to coma if uncorrected, without the presence of ketosis or acidosis. These patients have sufficient circulating insulin to prevent lipolysis and ketosis. Pathophysiology of DKA and HNC are given in Figure 6.
Figure 6: Pathophysiology of DKA and HNC. (Source: English and Williams. 2004).

CHRONIC COMPLICATIONS OF DIABETES MELLITUS

Diabetes mellitus is one of the most common chronic diseases worldwide and is associated with an increased morbidity and mortality. Diabetes is characterized by chronic hyperglycemia and alterations of cellular homeostasis, which lead to diffuse vascular damage. Generally, the injurious effects of hyperglycemia are separated into macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and micro vascular complications (diabetic nephropathy, neuropathy, and retinopathy).

The microvascular complications of diabetes, resulting from a damage of the microvasculature of the kidney, retina and neurons, include retinopathy, nephropathy and neuropathy (Figure 7). As a consequence of microvascular pathology, diabetes is an important determinant of blindness, end-stage renal disease and a variety of debilitating neuropathies. In addition, diabetes is associated with cardiovascular disease, which is an important contributor to the overall morbidity and mortality associated with this condition (Chiarelli and Marcovechio., 2013).
Several risk factors are implicated in the pathogenesis of diabetic complications and they can be either modifiable (glycemic control, hypertension, dyslipidemia, diet, smoking) or non-modifiable (diabetes duration, age at onset, puberty, genes). The pathogenesis of diabetic vascular complications is complex, with the involvement of several mechanisms and a clear contribution of genetic factors. Hyperglycemia is a key determinant of vascular complications of diabetes and there is extensive evidence showing that both acute and chronic hyperglycemia has a deleterious effect. Hyperglycemia contributes to the development of vascular complications through several mechanisms: activation of the polyol and hexosamine pathways, activation of protein kinase C, increased oxidative stress, increased production of advanced glycation end-products, increased synthesis of growth factors, cytokines and angiotensin II (Jeong and King, 2011). These factors can, in turn, induce a diffuse endothelial dysfunction and contribute to the progressive development of micro and macrovascular complications and multiorgan damage (Figure 8). Growing evidence suggests that increased oxidative stress, induced by several hyperglycemia-activated pathways, is a key factor in the pathogenesis of endothelial dysfunction and vascular disease (Chiarelli and Marcovecchio, 2013).
Several mitochondrial and other intracellular pathways are implicated in the increased production of oxidants, which is often associated with reduced antioxidant defences. In addition, recent studies suggest the involvement of epigenetic mechanisms as well as of micro RNAs in the pathogenesis of diabetic complications. The understanding and characterization of the molecular mechanisms underlying the development of vascular complications of diabetes is of paramount importance as this could help the development of better preventive and treatment strategies (Chiarelli and Marcovecchio., 2013).

**MICROVASCULAR COMPLICATIONS**

**Diabetic retinopathy**

One of the most common and distressing complications associated with diabetes is diabetic retinopathy (DR). The term retinopathy refers to any abnormal vascular changes in the retina, which, as a result of the associated pathology, can lead to loss of vision and even blindness (Kohner et al., 1996; Arun et al., 2003). Diabetic retinopathy is a chronic progressive, potentially sight-threatening disease of the retinal microvasculature associated with the prolonged hyperglycemia and other conditions linked to diabetes mellitus such as hypertension. Diabetic retinopathy is a potentially blinding disease in which the threat to sight comes through
two main routes: growth of new vessels leading to intraocular hemorrhage and possible retinal detachment with profound global sight loss, and localized damage to the macula / fovea of the eye with loss of central visual acuity (Wu et al., 2013).

Diabetic retinopathy is one of the most important causes of visual loss worldwide, and is the principal cause of impaired vision in patients between 20 to 74 years of age. Diabetic retinopathy can progress from non proliferative abnormalities to pre proliferative and finally to proliferative diabetic retinopathy (Fong et al., 2004). In the UK over recent years, there have been many significant advances in diabetes care; however, retinopathy still remains the leading cause of blindness in people of working age (Diabetes UK, 2010). The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on the duration and severity of hyperglycemia (Figure 9) (Michael and Fowler, 2008).

**Figure 9. Diabetic Retinopathy. (Source: Michael and Fowler, 2008)**

Risk factors associated with retinopathy

According to Emanuele et al., (2005), Shotliff and Duncan (2005) and The Royal College of Ophthalmologists (2012), some of the risk factors associated with retinopathy are:

- Long duration of diabetes.
- Suboptimal glycemic control.
- Hypertension.
- Nephropathy.
- Minority ethnic origin.
- Pregnancy can be associated with a rapid progression of retinopathy.
Although there appears to be no clear relationship between smoking and DR, in some people with Type 1 diabetes, smoking has been shown to be associated with microangiopathy, particularly when complications occur early in the course of their diabetes (Moss et al., 1991). It is, of course, good practice to ensure that all individuals are discouraged from smoking (Li et al., 2010).

The pathogenesis of diabetic retinopathy.

Diabetic retinopathy is clinically defined, diagnosed and treated based on the extent of retinal vascular disease exclusively. Three distinct forms of diabetic retinopathy are described:

1. Macular edema, which includes diffuse or focal vascular leakage at the macula;
2. Progressive accumulation of blood vessel change that includes micro aneurysms, intra retinal hemorrhage, vascular tortuosity and vascular malformation (together known as non proliferative diabetic retinopathy) that ultimately leads to abnormal vessel growth (proliferative diabetic retinopathy)
3. Retinal capillary closure, a form of vascular change detected on fluorescein angiography, which is also well recognized as a potentially blinding complication of diabetes but currently has no treatment options (Boyd et al., 2013).

Hyperglycemia appears to be a critical factor in the etiology of diabetic retinopathy and initiates downstream events including: basement membrane thickening, pericyte drop out and retinal capillary non-perfusion. More recently, focus has been directed to the molecular basis of the disease process in diabetic retinopathy. Of particular importance in the development and progression of diabetic retinopathy is the role of growth factors (eg. vascular endothelial growth factor, placenta growth factor and pigment epithelium-derived factor) together with specific receptors and obligate components of the signal transduction pathway needed to support them (Cai and Boulton., 2002) (Figure 10). Diabetic retinopathy is considered as a condition that affects the small blood vessels in the retina and is defined in terms of vascular endpoints (The Royal College of Ophthalmologists, 2012).
Diabetic Nephropathy

Diabetic nephropathy also known as Kimmelstiel-Wilson syndrome, or nodular diabetic glomerulosclerosis (Held et al., 1991) and intercapillary glomerulonephritis, is a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli. It is characterized by nephrotic syndrome and diffuse glomerulosclerosis. It is due to the long standing diabetes mellitus, and is a prime indication for dialysis in many Western countries. Diabetic nephropathy is a clinical syndrome characterized by excessive urinary albumin excretion, hypertension, and renal insufficiency. Normal urinary albumin excretion is less than 30 mg/24 h. Abnormal albumin excretion is defined as either microalbuminuria (30-299 mg/24 h) or macroalbuminuria (>300 mg/24 h (>300 mg/24 h) (American Diabetes Association., 2004).

Microvascular complications, including nephropathy, develop some years after the onset of diabetes. Genetic background is likely to be important in determining susceptibility to diabetic nephropathy, but exposure of tissues to chronic hyperglycemia is the main initiating factor. Diabetic nephropathy is major microvascular complication of diabetes, a leading cause of end-
stage renal disease and is associated with increased cardiovascular mortality (Gariani et al., 2012) Diabetic nephropathy is a clinical syndrome characterized by the occurrence of persistent albuminuria in concomitance with Type 1 and Type 2 diabetes.

The description of albumin in the urine as a sign of serious kidney diseases in 1836 by Bright, physician to Guy's Hospital, marked the advent of clinical nephrology. This observation, together with earlier ones by Cotunnius in 1770 and Rollo in 1798 that urine of some diabetics contained proteins, led Rayer in 1840 to postulate that diabetes might cause a form of "Bright's disease". Indeed various epidemiologic studies have demonstrated that about 20-40% of diabetic subjects will develop proteinuria and progressive renal failure on an average of 15-20 years after the onset of diabetes (Parchwani et al., 2012). The prognosis of these patients is poor and without renal support therapy, the mean survival after the onset of clinical proteinuria is only 5 years (Collins et al., 2012). Aside from the personal and domestic tragedy, the cost of caring for the diabetic patient in end stage renal disease is enormous. Analyses of the WHO Renal Data System demonstrated a dramatic increase in the incidence of ESRD that is caused by diabetes (Wang and Sarah., 2006). Between 1999 and 2005 diabetes was responsible for > 44% of all new cases of ESRD. Thus, whereas the population with diabetes grew 40% between 1984 and 1996, the number of people who initiated treatment for ESRD as a result of diabetes increased by 400%. Between 1996 and 2005, the annual incidence grew by another 37%. On the basis of these data, it is expected that the burden of diabetic nephropathy will increase further in the next years, although perhaps at a less accelerated growth rate. Caring for these patients is a formidable task since they are usually beset by numerous other diabetic complications. Successful management requires an understanding of the molecular mechanism and natural history of the condition.

The pathogenesis of diabetic nephropathy

The most problematic issue in clinical nephrology is the relentless and progressive increase in patients with ESRD (end-stage renal disease) worldwide. The impact of diabetic nephropathy on the increasing population with CKD (chronic kidney disease) and ESRD is enormous. Three major pathways showing abnormality of intracellular metabolism have been identified in the development of diabetic nephropathy: (i) the activation of polyol and PKC (protein kinase C) pathways; (ii) the formation of advanced glycation end-products; and (iii) intraglomerular hypertension induced by glomerular hyper filtration. Upstream of these three
major pathways, hyperglycemia is the major driving force of the progression to ESRD from diabetic nephropathy. Downstream of the three pathways, micro inflammation and subsequent extracellular matrix expansion are common pathways for the progression of diabetic nephropathy (Figure 11).

In recent years, many researchers have been convinced that the inflammation pathways play central roles in the progression of diabetic nephropathy, and the identification of new inflammatory molecules may link to the development of new therapeutic strategies. Various molecules related to the inflammation pathways in diabetic nephropathy include transcription factors, pro-inflammatory cytokines, chemokines, adhesion molecules, toll-like receptors, adipokines and nuclear receptors, which are candidates for the new molecular targets for the treatment of diabetic nephropathy. Understanding of these molecular pathways of inflammation would translate into the development of anti-inflammation therapeutic strategies (Wada and Makino, 2013).

**Figure 11. Inflammatory pathways in the pathogenesis of diabetic nephropathy.**

Diabetic Neuropathy

The term diabetic neuropathy refers to a set of clinical conditions in patients with diabetes that share as common feature abnormalities in the structure and the function of peripheral nerves. Diabetic peripheral neuropathy (DPN) also known as Sensory motor polyneuropathy is one of the most prevalent. More than 80% of amputations occur after foot ulceration or injury, which can result from diabetic neuropathy (Boulton et al., 2005). The most common form of DPN involves the somatic nervous system; the autonomic nervous system may be affected in some patients, (Boulton et al., 2005). Diabetic neuropathy is mainly categorized as diffuse (anatomically symmetrical) or focal (anatomically asymmetrical).

The pathogenesis of diabetic neuropathy

In the development of neuropathy, the hyperglycemic state leads to an increase in action of the enzymes aldose reductase and sorbitol dehydrogenase. This results in the conversion of intracellular glucose to sorbitol and fructose. The accumulation of these sugar products results in a decrease in the synthesis of nerve cell myoinositol, required for normal neuron conduction. Additionally, the chemical conversion of glucose results in a depletion of nicotinamide adenine dinucleotide phosphate stores, which are necessary for the detoxification of reactive oxygen species and for the synthesis of the vasodilator nitric oxide. There is a resultant increase in oxidative stress on the nerve cell and an increase in vasoconstriction leading to ischemia, which will promote nerve cell injury and death. Hyperglycemia and oxidative stress also contribute to the abnormal glycation of nerve cell proteins and the inappropriate activation of protein kinase C, resulting in further nerve dysfunction and ischemia (Figure 12).
Figure 12: Showing the pathogenesis of diabetic neuropathy (Boulton, 2005).

MACROVASCULAR COMPLICATIONS

Macrovascular complications associated with diabetes include cardiovascular, cerebrovascular, and peripheral arterial diseases. Damage to the larger arteries leading to the brain (leading to brain stroke) or to the heart (leading to coronary heart disease) or to the legs and feet (leading to peripheral vascular disease) leads to macrovascular complications including diseases of coronary arteries, peripheral arteries, and carotid vessels. Most patients with diabetes die from complications of atherosclerosis. Clinical manifestations of atherosclerosis occur primarily in 3 vascular beds: coronary arteries, lower extremities and extra cranial carotid arteries. The central pathological mechanism in macrovascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls throughout the body. Atherosclerosis is thought to result from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system. In response to endothelial injury and inflammation, oxidized lipids from LDL particles accumulate in the endothelial wall of arteries. Angiotensin II may promote the oxidation of such particles. Monocytes then infiltrate the arterial wall and differentiate into macrophages, which accumulate oxidized lipids to form foam cells. Once formed, foam cells stimulate macrophage proliferation and attraction of T-lymphocytes. T-lymphocytes, in turn,
induce smooth muscle proliferation in the arterial walls and collagen accumulation. The net result of the process is the formation of a lipid-rich atherosclerotic lesion with a fibrous cap. Rupture of this lesion leads to acute vascular infarction (Boyle, 2007). In addition to atheroma formation; there is strong evidence of increased platelet adhesion and hyper coagulability in Type 2 diabetes. Impaired nitric oxide generation and increased free radical formation in platelets, as well as altered calcium regulation, may promote platelet aggregation. Elevated levels of plasminogen an activator inhibitor Type 1 may also impair fibrinolysis in patients with diabetes. The combination of increased coagulability and impaired fibrinolysis likely further increases the risk of vascular occlusion and cardiovascular events in Type 2 diabetes (Beckman et al., 2002).

**Cardiovascular disease**

CVD is the primary cause of death in people with either Type 1 or Type 2 diabetes (Laing et al., 2003; Paterson et al., 2007). People with diabetes are 2 to 4 times more likely to develop CVD than those without diabetes (National Diabetes Fact Sheet., 2005). Among people with Type 2 diabetes, women may be at higher risk for coronary heart disease than men. The presence of microvascular disease is also a predictor of coronary heart events (Avogaro et al., 2007). There are several risk factors that may contribute to the development of CHD, including lifestyle (e.g. cigarette smoking and diet), hyperglycemia, hypertension, and high cholesterol. Additional mechanisms that contribute to the increased risk of CHD and worse outcomes in persons with diabetes include endothelial dysfunction, hypercoagulability, impaired fibrinolysis, platelet hyperaggregability, oxidative stress, sympathovagal imbalance, and glucose toxicity (Haffner, 2005).

**The pathogenesis of CVD**

Cardiovascular diseases are the most prevalent cause of morbidity and mortality among patients with Type 1 or Type 2 diabetes (Orasanu and Plutzky, 2009; Laing et al., 2003). In general, patients with diabetes aggregate other co-morbidities such as obesity, hypertension, and dyslipidemia which also contribute to increase the risk for CVD (Al Ghatrif et al., 2011). Diabetes, obesity, and insulin resistance are associated with subclinical inflammation characterized by over expression of cytokines produced by adipose tissue, activated macrophages, and other cells (Hotamisligil, 2006; Shoelson et al., 2006). Inflammatory
mediators, such as TNF-α, interleukin-1 (IL-1), IL-6, leptin, resistin, MCP-1, plasminogen activator inhibitor-1 (PAI-1), C-reactive protein (CRP), fibrinogen, angiotensin, visfatin, retinol binding protein-4, and adiponectin are involved in signaling pathways, in insulin action, and perpetuation of inflammatory response (Shoelson et al., 2006). These cytokines are involved in the chronic inflammatory process of the vessels wall, promoting lipid accumulation with consequent development of atherosclerosis and CVD (Vicenova et al., 2009) (Figure 13).

**Figure 13: Showing the Pathogenesis of cardiovascular disease in diabetes.**

(Shoelson et al., 2006).

![Diagram of cardiovascular disease in diabetes](image)

The mechanisms involved in the pathogenesis of cardiovascular disease in diabetes comprehend epigenetic changes and intracellular metabolic changes that result in oxidative stress, low-grade inflammation, and endothelial dysfunction. CRP: C-reactive protein; FFA: free fatty acids; INOS: inducible nitric oxide synthase; IL-1: interleukin 1; IL-6: interleukin 6; MCP-1: monocyte chemoattractant molecule 1; MMP: matrix metalloprotease; NF-κB: nuclear factor kappa-β; PAI-1: plasminogen activator inhibitor-1; VCAM-1: vascular cell adhesion molecule-1; VEGF: vascular endothelial growth factor; TNF-α: Tumor necrosis factor-α; INF-γ: Interferon-γ (Vicenova et al., 2009).
Atherosclerosis is a complex multifactorial disease, and the acceleration of atherosclerosis in diabetes may be explained by several conditions including hyperglycemia, increased oxidative stress, advanced glycation end products (AGE), dyslipidemia, autonomic imbalance, hyperinsulinemia, inflammatory markers excess, and genetic variables (Renard et al., 2004; Ferrarezi et al., 2007; Ait-Oufella et al., 2011). Several cytokines described to be related with insulin resistance are also involved with the development of atherosclerosis and CVD. TNF-α and other cytokines, FFA and ROS, activate inflammatory pathways and promote the expression of numerous genes involved in insulin resistance (Hotamisligil, 2006; Shoelson et al., 2006; Wellen and Hotamisligil, 2005). The classical risk factors for the development of CVD in subjects with diabetes are the presence of poor glycemic control, obesity, dyslipidemia, and hypertension.

**Cerebrovascular disease**

Cerebrovascular disease is a term encompassing many disorders that affect the blood vessels of the central nervous system. These disorders result from either inadequate blood flow to the brain (i.e., cerebral ischemia) or from hemorrhages into the parenchyma or subarachnoid space of the central nervous system. Various terms have been used to describe cerebrovascular events. For example, the term transient ischemic attack (TIA) describes the clinical condition in which a patient experiences a temporary focal neurologic deficit such as slurred speech, aphasia, weakness or paralysis of a limb, or blindness. These symptoms are rapid in onset, lasting, 24 hours (usually 2 to 15 minutes). Reversible ischemic neurologic deficit is similar to a TIA; however, the deficit improves over no more than 72 hours and may not completely resolve. Cerebral infarction is a neurologic event causing permanent damage. Cerebral hemorrhage is a cerebrovascular disorder that involves escape of blood from blood vessels into the brain and its surrounding structures. There are 700,000 new or recurrent cerebrovascular events per year. The incidence of stroke is significantly greater among blacks compared with whites (Welty., 2001). Sudden confusion, loss of coordination, unilateral weakness, and numbness are warning signs of a cerebrovascular event.
Pathophysiology of cerebrovascular disease

1. Atherosclerosis and subsequent plaque formation results in arterial narrowing or occlusion and is the most common cause of arterial stenosis.

2. Thrombus formation is most likely to occur in areas where atherosclerosis and plaque deposition have caused the greatest narrowing of vessels.

3. Platelet aggregation
   a. exposed sub endothelium after injury to vessel
   b. vessel collagen is exposed to blood triggering "activation" of platelets
   c. release of ADP from activated platelets causes platelet aggregation
   d. consolidation of platelet-plug by RBCs, coagulation factors, and formation of fibrin network
   e. Thromboxane A2 (TX A2) is produced by platelets and endothelium promoting platelet aggregation and vasoconstriction

4. Coagulation Cascade
   a. a series of enzyme complexes located on the surface of platelets and endothelium which lead to thrombin production
   b. Thrombin (IIa) then converts Fibrinogen to Fibrin

Peripheral Arterial Disease

Peripheral arterial disease (PAD) is an atherosclerotic occlusive disease. It is the major risk factor for lower extremity amputations. The abnormal metabolic state accompanying diabetes results in changes in the state of arterial structure and function predisposing people to PAD (Creager and Libby, 2001). The risk of development of PAD increases 3 to 4 fold in patients with diabetes mellitus (Murabito et al., 1997). Risk factors for the development of PAD include diabetes, hypertension, hyperlipidemia, cigarette smoking, and age. In people with diabetes, the risk of PAD is increased by age, duration of diabetes, and presence of peripheral neuropathy. Elevated levels of C-reactive protein (CRP), fibrinogen, homocysteine, apolipoprotein B and plasma viscosity are potential risk factors for PAD.
List of complications linked to badly controlled diabetes

- **Eye complications** - glaucoma, cataracts, diabetic retinopathy, and some others.
- **Foot complications** - neuropathy, ulcers, and sometimes gangrene which may require that the foot be amputated
- **Skin complications** - people with diabetes are more susceptible to skin infections and skin disorders
- **Heart problems** - such as ischemic heart disease, when the blood supply to the heart muscle is diminished
- **Hypertension** - common in people with diabetes, which can raise the risk of kidney disease, eye problems, heart attack and stroke
- **Mental health** - uncontrolled diabetes raises the risk of suffering from depression, anxiety and some other mental disorders
- **Hearing loss** - diabetic patients have a higher risk of developing hearing problems
- **Gum disease** - there is a much higher prevalence of gum disease among diabetes patients
- **Gastro paresis** - the muscles of the stomach stop working properly
- **Ketoacidosis** - a combination of ketosis and acidosis; accumulation of ketone bodies and acidity in the blood.
- **Neuropathy** - diabetic neuropathy is a type of nerve damage which can lead to several different problems.
- **HHNS (Hyperosmolar Hyperglycaemic Nonketotic Syndrome)** - blood glucose levels shoot up too high, and there are no ketones present in the blood or urine. It is an emergency condition.
- **Nephropathy** - uncontrolled blood pressure can lead to kidney disease
- **PAD (peripheral arterial disease)** - symptoms may include pain in the leg, tingling and sometimes problems walking properly
- **Stroke** - if blood pressure, cholesterol levels, and blood glucose levels are not controlled, the risk of stroke increases significantly
- **Erectile dysfunction** - male impotence.
- **Infections** - people with badly controlled diabetes are much more susceptible to infections
- **Healing of wounds** - cuts and lesions take much longer to heal.
TREATMENT OF DIABETES MELLITUS

The aim of the treatment is primarily to save life and alleviate symptoms. Secondary aims are to prevent long term diabetic complications and, by eliminating various risk factors, to increase longevity. The first aim is not difficult to attain and in some elderly patients or those who lack motivation it is the only aim (Watkins et al., 1990). The care of diabetes on self management is based on the patient’s clinical status and his/her ability to participate in self-care. Insulin replacement therapy is the mainstay for patients with Type 1 DM while diet and lifestyle modifications are considered the cornerstone for the treatment and management of Type 2 DM. Insulin is also important in Type 2 DM when blood glucose levels cannot be controlled by diet, weight loss, exercise and oral medications. Oral hypoglycaemic agents are also useful in the treatment of Type 2 DM.

The major approaches of the treatment of diabetes are:

1. Diet (combined with exercise)
2. Oral hypoglycaemic agents
3. Insulin therapy
4. Herbal therapy

1. Dietary management

Diet is a basic part of management in every case. Treatment cannot be effective unless adequate attention is given to ensuring appropriate nutrition. Ideally, the initial management of NIDDM should be based on dietary therapy combined with increased physical activity, if possible. However, pharmacologic therapy (oral hypoglycemic drugs or insulin) may be considered in the presence of marked hyperglycemia.

Dietary treatment should aim at:

- Ensuring weight control
- Providing nutritional requirements
- Allowing good glycemic control with blood glucose levels as close to normal as possible.
- Correcting any associated blood lipid abnormalities
- Ensuring consistency and compatibility with other forms of treatment if used, for example oral agents or insulin.
The following principles are recommended as dietary guidelines for people with Diabetes.

- Fat should provide 25-35% of total intake of calories but saturated fat intake should not exceed 10% of total energy. Cholesterol consumption should be restricted and limited to 300mg or less daily.
- Protein intake can range between 10-15% total energy (0.8-1 g/kg of desirable body weight). Requirements increase for children and during pregnancy. Protein should be derived from both animal and vegetable sources.
- Carbohydrates provide 50-60% of total caloric content of the diet. Although it has been traditionally recommended that carbohydrates should be complex and high in fibre, more emphasis should be placed on the total amount of carbohydrates consumed than the source of carbohydrate.
- Excessive salt intake is to be avoided. It should be particularly restricted in people with hypertension and those with nephropathy.
- Artificial sweeteners are to be used in moderation. Nutritive sweeteners (sorbitol and fructose) should be restricted.

The same precautions regarding alcohol intake that apply to the non diabetic population also apply to people with diabetes. Additionally, however, alcohol tends to increase the risk of hypoglycemia in those taking antidiabetic drugs and should be particularly avoided in those with lipid abnormalities and patients with neuropathy. Except in special conditions like pregnancy and lactation, routine vitamin and mineral supplementation is generally not needed in people with a well balanced diet. There is, at present, no definite evidence to confirm that such treatment has any benefits.

**Exercise**

Physical activity promotes weight reduction and improves insulin sensitivity, thus lowering blood glucose levels. Together with dietary treatment, a programme of regular physical activity and exercise should be considered for each person. Such a programme must be tailored to the individual’s health status and fitness.

**2. Oral hypoglycemic agents**

Oral hypoglycemic agents (OHA’s) are considered only after a regimen of dietary treatment combined with exercise has failed to achieve the therapy targets set.
Currently, there are five distinct classes of OHAs available

1. Sulfonylureas/Sulphonylureas (SUs)
2. Meglitinides
3. Biguanides
4. Thiazolidinediones (TZDs)/glitazone
5. \(\alpha\)-glucosidase inhibitors

Each class displays unique pharmacological properties. Mechanisms of action of five classes of oral hypoglycemic agents are summarized in Figure 14.

**Figure 14: Summary of the mechanisms of action of the oral hypoglycemic agents**
(Source: Boavida et al., 2007)

**SULFONYLUREAS**

Sulfonylureas are frequently classified as either 1\textsuperscript{st} generation or 2\textsuperscript{nd} generation agents. First generation sulfonylureas (acetohexomide, chlorpropamide, tolazamide and tolbutamide) possess a lower binding affinity for the ATP-sensitive potassium channel, their molecular target, and thus require higher doses to achieve efficacy, increasing the potential for adverse events. In addition, the plasma half-life of 1\textsuperscript{st} generation sulfonylureas is extended compared to the 2\textsuperscript{nd} generation agents. 2\textsuperscript{nd} generation sulfonylureas including glyburide (glibenclamide), glipizide, and glimepiride are now widely used. The 2\textsuperscript{nd} generation sulfonylurea’s are much more potent compounds (~100 fold), with a more rapid onset of action, and generally have shorter plasma half-lives and longer duration of action compared to the 1\textsuperscript{st} generation agents.
**Mechanism of action**

Sulfonylureas are insulin secretogogues, since they control blood glucose levels by directly stimulating first-phase insulin secretion in the pancreatic β-cells. Mitochondrial glucose metabolism leads to ATP generation and increases the intracellular ratio of ATP/ADP, which results in the closure of the ATP-sensitive potassium channel (K⁺-ATP; a 140 kDa membrane protein) on the plasma membrane of β-cells. Closure of this channel depolarizes the membrane and triggers the opening of voltage-sensitive calcium channels, leading to the rapid influx of calcium. Increased intracellular calcium causes an alteration in the cytoskeleton, and stimulates translocation of insulin-containing secretory granules to the plasma membrane and the exocytotic release of insulin.

The K⁺-ATP channel is comprised of two subunits. One subunit contains the cytoplasmic binding sites for both sulfonylureas and ATP, and is designated as the sulfonylurea receptor type 1 (SUR1). The other subunit is the potassium channel, which acts as the pore-forming subunit (Inagaki et al., 1995). Either an increase in the ATP/ADP ratio or ligand binding (by sulfonylureas, meglitinides) to SUR1 results in the closure of the K⁺-ATP channel and insulin secretion (**Figure 15**). Studies comparing sulfonylureas and non-sulfonylurea insulin secretogogues have identified several distinct binding sites on the SUR1 that cause channel closure.

**Figure 15: Proposed Mechanistic action of Sulfonylureas, (Inagaki et al., 1995).**
**Efficacy**

All sulfonylureas are equally effective in terms of their hypoglycemic potency, although a recent trial has indicated that glimepiride (Amaryl) may be slightly more efficacious than the others (Ahmann and Riddle, 2000).

**Side effects**

The most common side effect of sulfonylurea is hypoglycaemia, which though usually mild to moderate, can cause fatal complication (Ferner and Neil., 1988; Seltzer., 1989). In the United Kingdom Prospective Diabetic Study (UKPDS) (UK Prospective Diabetes Study Group., 1998) the rates of any hypoglycemic symptoms were 11% for chlorpropamide, 17.7% for glibenclamide, 36.5% for insulin, and 1.2% for lifestyle management. Long-lasting and serious hypoglycaemia occurs more often with long acting sulfonylureas, such as glibenclamide and chlorpropamide than with short-acting ones, such as glipizide and tolbutamide (Swedish Board of Health and Welfare., 1985) Weight gain is a frequent complication of sulfonylurea treatment and well-controlled studies have found that the mean yearly increase in body weight was 2.8 kg (Campbell and Howlett., 1995) In UKPDS, patients receiving sulfonylureas had a net increase in weight of 3 kg compared to conventionally treated patients (The UKPDS Group., 1995).

Other effects may include gastrointestinal disturbances and headache. Hypersensitivity reactions are uncommon but may occur in the first 6-8 weeks of therapy and include transient rashes, fever, and jaundice. Blood disorders are rare, but include thrombocytopenia, agranulocytosis, and aplastic and haemolytic anaemias. About 10-15% of patients on chlorpropamide develop an alcohol flushing reaction similar to that caused by disulfiram (disulfiram-Antibuse reaction). Chlorpropamide may also induce hyponatremia by potentiating the effects of antidiuretic hormone on the renal collecting duct, (Paice et al., 1985) which may occur in about 5% of all patients; it is less frequent with glyburide and glipizide.

**MEGLITINIDES**

The meglitinides are a new class of drugs developed to improve early-phase insulin secretion, which is one of the earliest pathophysiological manifestations of Type 2 DM. These are derived from the meglitinide portion of sulfonylureas. Examples of this group are repaglinide and nateglinide. Another meglitinide known as mitiglinide is undergoing clinical trials. Repaglinide, a benzoic acid derivative introduced in 1998, was the first member of the
meglitinide class. Nateglinide is a derivative of the amino acid D-phenylalanine and was introduced to the market in 2001.

**Mechanism of action**

The meglitinides act on β-cell receptors to stimulate insulin secretion by binding to the sulfonylurea receptor subunit and closing the K⁺-ATP channel (Hu et al., 2000), but probably at a site distinct from that of the sulfonylurea receptor (Fuhlendorff et al., 1988). Repaglinide and Nateglinide does not stimulate insulin secretion in the complete absence of glucose and its action is usually confined to intermediate concentrations of glucose i.e. 180 mg/dL (10 mmol/L) glucose. These properties account for the low risk of hypoglycaemia seen with repaglinide in contrast to the sulfonylureas.

**Adverse effects**

In 1-year trials, the most common adverse events reported in repaglinide recipients (n = 1,228) were hypoglycemia (16%), upper respiratory tract infection (10%), rhinitis (7%), bronchitis (6%) and headache (9%). Weight gain does occur in patients treated with repaglinide, but the magnitude is significantly less compared to treatment with glyburide (Marbury et al., 1999; Damsbo et al., 1999). The most common adverse effects are nausea, diarrhoea, dizziness, and lightheadedness with nateglinide and incidence of mild hypoglycemia is lower than for repaglinide and no reports of severe hypoglycemia and weight gain. Repaglinide and nateglinide should be used cautiously in patients with hepatic insufficiency. They are contraindicated in severe hepatic impairment, pregnancy and breastfeeding.

**BIGUANIDES**

Metformin (Glucophage) and phenformin were introduced in 1957 and buphormin was introduced in 1958. They were widely used in Europe for treating type 2 diabetes for nearly 20 years. The latter two were withdrawn in many countries in the 1970s because of an association with fatal lactic acidosis (Schafer., 1983). Metformin (dimethyl biguanide; Glucophage) is a synthetic analog of the natural product guanidine. Metformin has a very low rate of lactic acidosis compared to phenformin and has been widely used in Europe, Canada, Middle East and other countries; it became available in the United States in 1995. Metformin is recommended as a first-line therapy in newly diagnosed individuals, and can be used in combination with an insulin secretagogue (sulfonylurea or meglitinide), thiazolidinedione, α-glucosidase inhibitor, exenatide, DPP-4 inhibitor or insulin (DeFronzo., 2000; Bolen et al., 2007).
Mechanism of action

The primary effect of metformin is the suppression of basal hepatic glucose production, thereby reducing fasting plasma glucose. The molecular target of metformin action still awaits identification. Metformin does not stimulate insulin secretion; in contrast, metformin reduces fasting plasma insulin and improves whole-body insulin-stimulated glucose metabolism (insulin sensitivity) (Bailey and Turner, 1996; Cusi and DeFronzo, 1998). Recent in vitro and in vivo evidence has shown that metformin activates the AMP-activated protein kinase (AMPK), a major cellular regulator of lipid and glucose metabolism (Hardie et al., 1998). As a result, acetyl-CoA carboxylase activity was reduced, fatty acid oxidation was induced (due to decreased malonyl-CoA), and the expression of lipogenic enzymes along with SREBP-1, a key lipogenic transcription factor was suppressed (Zhou et al., 2001). The use of a novel AMPK inhibitor indicated that AMPK activation was required for the inhibitory effect of metformin on glucose production in hepatocytes. In isolated rat skeletal muscles, metformin stimulated glucose uptake coincident with AMPK activation.

Adverse effects

Approximately one-third of patients on metformin will have transient nausea, anorexia or diarrhoea, abdominal discomfort, and metallic taste. Intestinal absorption of vitamin B12 and folate is often decreased during chronic metformin therapy. Calcium supplements reverse the effect of metformin on vitamin B12 absorption (Bauman et al., 2000). Other adverse effects reported are headache, agitation, dizziness and tiredness. Lactic acidosis is a rare but serious, and it is estimated to have an incidence of 0.03 per 1000 patient/years (Bailey and Turner, 1996). Metformin is contraindicated in patients with impaired renal, respiratory or hepatic function, cardiac failure, or a history of alcohol abuse.

α-Glucosidase inhibitors

Acarbose, miglitol and voglibose are members of the α-glucosidase inhibitor class of oral anti-hyperglycemic compounds that function by blocking the enzymatic degradation of complex carbohydrates in the small intestine (Magnier and Amatruda, 2000) and decrease the postprandial rise in plasma glucose, thus reproducing the effect of a low glycemic index/high fiber diet. These drugs have an excellent safety profile.
Mechanism of action

Acarbose, the first α-glucosidase inhibitor discovered, is a nitrogen-containing pseudotetrasaccharide of microbial origin, while miglitol is a synthetic analog of 1-deoxy nojirimycin. The mechanism of action of these inhibitors is similar but not identical. They bind competitively to the oligosaccharide binding site of the α-glucosidase enzymes, thereby preventing enzymatic hydrolysis. Acarbose binding affinity for the α-glucosidase enzymes is: glycoamylase > sucrase > maltase > dextranase (Puls., 1996). Acarbose has little affinity for isomaltase and no affinity for the β-glucosidase enzymes, such as lactase. Miglitol is a more potent inhibitor of sucrase and maltase acarbose, has no effect on α-amylase, but does inhibit intestinal isomaltose (Lebovitz., 1998).

Adverse effects

The major side effects of the α-glucosidase inhibitors are related to gastrointestinal disturbances. These occur in approximately 25-30% of diabetic patients, the delay in carbohydrate digestion and their accumulation in the lower gastrointestinal tract increases the amount of fermentable carbohydrate reaching the colon. This results in dose-related flatulence, diarrhoea, and abdominal bloating. Acarbose is contraindicated in patients with inflammatory bowel disease, cirrhosis, or elevated plasma creatinine (>177 μmol/L). This class of drugs is associated with dose-dependent hepatotoxicity, and serum transaminase levels require monitoring for patients receiving high doses (>200 mg three times daily).

THIAZOLIDINEDIONES

Pioglitazone, rosiglitazone or avandia and troglitazone are members of the thiazolidinedione class of insulin sensitizing compounds originally discovered and characterized for their glucose- and lipid-lowering activity (Sohda et al., 1982; 1995). A thiazolidine-2, 4-dione structure is common to all agents. These compounds decrease insulin resistance and enhance the biological response to endogenously produced insulin, as well as insulin administered by injection (Mudaliar and Henry, 2001; Foyt et al., 2000). Troglitazone was withdrawn from use because of its association with severe hepatic toxicity (Bae et al., 2003).
Mechanism of action

Pioglitazone and rosiglitazone are selective agonists for the peroxisome proliferator-activated receptor γ (PPARγ), a member of the superfamily of nuclear hormone receptors that function as ligand-activated transcription factors (Kliewer et al., 1999). In the absence of ligand, PPARs bind as heterodimers with the 9-cis retinoic acid receptor (RXR) and a multi-component co-repressor complex to a specific response element (PPRE) within the promoter region of their target genes (Olefsky and Saltiel., 2000). Once PPAR is activated by ligand, the co-repressor complex dissociates allowing the PPAR-RXR heterodimer to associate with a multi-component co-activator complex resulting in an increased rate of gene transcription. The target genes of PPARγ include those involved in the regulation of lipid and carbohydrate metabolism (Picard and Auwerx., 2002).

PPARγ is expressed chiefly in adipose tissue, and its expression in liver and skeletal muscle is low (Fajas et al., 1997). Thus, it is more likely that the primary effects of these drugs are on adipose tissue, followed by secondary benefits on other target tissues of insulin (Combs et al., 2002). The ability of rosiglitazone and pioglitazone to decrease circulating free fatty acids could lead to an improvement in insulin action in the periphery (de Souza et al., 2001). More recently, PPARγ agonists have been reported to increase the expression and circulating level of adiponectin (Acrp30), an adipocyte-derived protein with insulin sensitizing activity (Berg et al., 2001), in diabetic rodents (Combs et al., 2002) and in patients with type 2 diabetes (Yang et al., 2002).

Adverse effects

The major side effects of this class of drugs are edema, weight gain, decreased hematocrit and hemoglobin, and elevated (but reversible) alanine aminotransferase activity. Weight gain (dose-dependent) of 1-4kg after 6 months of treatment (Aronoff et al., 2000) and fluid retention that may be severe enough to exacerbate or precipitate heart failure (Idris et al., 2003), were observed with TZDs. The drugs also cause gastro-intestinal disturbances, anaemia, headache, visual disturbances, dizziness, haematuria, impotence; less commonly fatigue, insomnia, vertigo, hypoglycaemia and proteinuria. Rosiglitazone has not been shown to be hepatotoxic in premarketing trials; a few case reports have implicated it as a cause of acute hepatocellular injury (Dhawan et al., 2002). Rosiglitazone or Avandia is an anti-diabetic drug in the thiazolidinedione
class of drugs. It works as an insulin sensitizer, by binding a component in fat cells and making the cells more responsive to insulin. But, the Governments of US and European Union have banned the production and import of Rosiglitazone (Avandia) due to cardiac problems caused by Rosiglitazone.

3. INSULIN THERAPY

Insulin was discovered by Banting and Best in 1922 completely revolutionizing the treatment of diabetes mellitus. Progress has been made, in recent years, in the production, formulation and delivery of insulin preparations, as well as the development of insulin treatment regimens which maintain long-term normoglycaemia with a low risk of hypoglycemia. Insulin is the most potent glucose-lowering agent, with hypoglycemia being the only major dose-limiting factor. Insulin has progressively more side effects as the dose is increased and may be administered intravenously or intramuscularly. However for long-term treatment, subcutaneous route is preferred (Bastaki et al., 2005). Insulin significantly reduces glucose concentrations by suppressing hepatic glucose production, increasing postprandial glucose utilization and improving the abnormal lipoprotein that is characteristic of insulin resistance. Insulin therapy may also decrease or eliminate the effects of glucose toxicity by reducing hyperglycemia to improve insulin sensitivity and β-cell secretory function (Soeborg et al., 2009). With the advent of recombinant DNA technology, more adaptable forms of insulin analogues have been designed. The subsequent availability of rapid acting (insulin lispro, insulin aspart) and long acting (insulin glargine and detemir insulin) insulin analogues for meal and basal requirements offer both individual and collective advantages (Yadav and Parakh, 2006; Monami et al., 2008). The development towards insulin delivery led to external continuous subcutaneous insulin infusion pumps, capable of achieving excellent metabolic control and reduced risk of hypoglycemia (Tripathi and Srivastava, 2006).

Adverse effects of insulin therapy

The most common adverse reactions to insulin are weight gain and hypoglycaemia (Henry et al., 1993; Kudlacek et al., 1992). Hypoglycaemia may result from an inappropriately large dose, from mismatch between the peak delivery of insulin and food intake or from superimposition of additional factors that increase sensitivity to insulin (adrenal insufficiency, pituitary insufficiency) or that increase insulin-independent glucose uptake (exercise). The more
vigorously the attempt to achieve euglycaemia, the more frequent the episodes of hypoglycaemia. In one clinical trial (DCCT), the incidence of hypoglycaemia reactions were three times higher in the intensive insulin therapy group than in the conventional therapy group (Diabetes Control and Complications Trial Research Group., 1993). Weight gain after starting insulin therapy for uncontrolled diabetes is an inevitable consequence and is the result of increased truncal fat and muscle bulk (Diabetes Control and Complications Trial Research Group., 1993; Yki-Jarvinen et al., 1999). This is also due to reduced energy losses through glycosuria. In this case physiological insulin regimens can help to minimize weight gain by reducing inappropriate insulinaemia and hypoglycaemia between meals and thus the need for snacks in both adults and children. In type 2 diabetes metformin can help limit weight gain when insulin is started (Yki-Jarvinen et al., 1999).

There has been a dramatic decrease in the incidence of resistance and allergic reactions to insulin with the use of human insulin or highly purified preparations of the hormone. Bovine insulin is especially prone to cause allergic reactions. These reactions still occur as a result of the small amounts of aggregated or denatured insulin in all preparations, to minor contaminants, or because of sensitivity to one of the components added to insulin in its formulation (protamine, Zn\textsuperscript{2+}, phenol, etc.). The most frequent allergic reactions were IgE-mediated local urticaria reactions which are extremely rare nowadays (Kahn and Rosenthal., 1979). Skin testing are useful, however many patients exhibit positive reaction to intradermal insulin without experiencing any adverse effect.

4. HERBAL THERAPY OF DIABETES MELLITUS

The art of herbal medicine is extremely ancient, probably predates modern Homo sapiens (Eisenberg et al., 2003). In ancient cultures, people methodically and scientifically collected information on herbs and developed well-defined herbal pharmacopoeias. The earliest recorded evidence of such efforts in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include Charak Samhita and Sushruta Samhita. Irrespective of the decline in use of herbal medicines, the importance of botanicals in the evolution of medicine remains unchallenged. Many drugs are developed with Phytochemicals or taking Phytochemicals as lead molecules. The valuable mainline drugs include digitalis, cinchona, taxol, ergotamine, morphine, cocaine, reserpine and numerous others.
Several pharmacopoeias have provided parameters to maintain quality and standardize procedures in identification/authentication of herbal inputs and their products. The European Pharmacopoeia 2002 has 174 monographs on herbal drugs and preparations. British Herbal Pharmacopoeia has 233 monographs, British Herbal Compendium has 84 monographs, United States Pharmacopoeia and the National Formulary has 28 official monographs of the most commonly used plants in the country. The countries with strong background of traditional medicine as China and India are leading. Chinese Pharmacopoeia 2000 has 992 monographs and Ayurvedic pharmacopoeia of India [API] has about 1000 single drugs and 8000 compound formulations of recognized merit used in India (Inamdar et al., 2007). The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world (Seth and Sharma, 2004).

The recorded use of herbal remedies for the treatment of diabetes mellitus goes back as far as the Ebrus Papyrus 1550 BC (Day., 1990). Recognition of the disease in early times is illustrated by an ancient Indian text (6 BC) in which Ayurvedic Physician Susruta described two forms of madhumeha or sweet urine – an “inherited” type which causes emaciation and a second type which affects individuals with sedentary habits and a tendency to over eat (Shanmugasundaram et al., 1983). Traditional medicine systems from around the world, particularly Arabia, China and the Indian subcontinent, have evolved a range of herbal treatments for diabetes (Nadkarni., 1982; Bailey and Day., 1989; Zhang and Xiao., 1993). Diabetes affects about 5% of the global population (Chakraborty and Rajagopalan., 2002) and management of diabetes without any side effects is still a challenge to the medical system (Kameswara Rao et al., 2003a).

Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (Venkatesh et al., 2003). In recent years, herbal medicines have started to gain importance as a source of hypoglycemic agents. Therefore, investigation on such agents from traditional medicinal plants has become more important (Suba et al., 2004a; WHO., 1980). India has a rich history of using various potent herbs and herbal
components for treating diabetes. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reported in numerous scientific journals.

In Central Drug Research Institute, Lucknow, India, more than 2000 plants have been evaluated for their blood sugar lowering activity. A number of excellent reviews on antidiabetic plants and active Phytochemicals have been published. Mukherjee (1981) reviewed about 40 plant species with hypoglycaemic activity. Ivorra et al., (1989) mentioned the antidiabetic activities of different plant products and their active ingredients.


There are many herbal remedies suggested for diabetes and diabetic complications in India. Medicinal plants form the main ingredients of these formulations. A list of such formulations is given in Table 3.
Table 3: Formulated Herbal Drugs with antidiabetic properties, (Modak et al., 2007).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Company</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabecon</td>
<td>Himalaya</td>
<td>Gymnema sylvestre, Pterocarpus marsupium, Glycyrrhiza glabra, Casearia esculenta, Syzygium cumini, Asparagus racemosus, Boerhavia diffusa, Sphaeranthus indicus, Tinospora cordifolia, Swertia chirata, Tribulus terrestris, Phyllanthus amarus, Gmelina arborea, Gossypium herbaceum, Berberis aristata, Aloe vera, Triphala, Commiphora wightii, shilajite, Momordica charantia, Piper nigrum, Ocimum sanctum, Abutilon indicum, Curcuma longa, Rumex maritimus</td>
</tr>
<tr>
<td>Diasulin</td>
<td>Himalaya</td>
<td>Cassia auriculata, Coccinia indica, Curcuma longa, Emblica officinalis, Gymnema sylvestre, Momordica charantia, Scoparia dulcis, Syzygium cumini, Tinospora- cordifolia, Trigonella foenum graecum</td>
</tr>
<tr>
<td>Pancreatic tonic180 cp</td>
<td>Ayurvedic herbal supplement</td>
<td>Pterocarpus marsupium, Gymnema sylvestre, Momordica charantia, Syzygium cumini, Trigonella foenum graceum, Azadirachta indica, Ficus racemosa, Aegle marmelos, Cinnamomum tamala</td>
</tr>
<tr>
<td>Ayurveda alternative herbal formula to Diabetes:</td>
<td>Chakrapani Ayurveda</td>
<td>Gurmar (Gymnema sylvestre) Karela (Momordica charantia) Pushkarmool (Inula racemosa) Jamun Gutli (Syzygium cumini) Neem (Azadirachta indica) Methika (Trigonella foenum graceum) Guduchi (Tinospora cordifolia)</td>
</tr>
<tr>
<td>Bitter gourd Powder</td>
<td>Garry and Sun natural Remedies</td>
<td>Bitter gourd (Momordica charantia)</td>
</tr>
<tr>
<td>Dia-care</td>
<td>Admark Herbals Limited</td>
<td>Sanjeevan Mool; Himej, Jambu beej, Kadu, Namejav, Neem chal.</td>
</tr>
<tr>
<td>Diabetes-Daily Care</td>
<td>Nature’s Health Supply</td>
<td>Alpha Lipoic Acid, Cinnamon 4% Extract, Chromax, Vanadium, Fenugreek 50% extract, Gymnema sylvestre 25% extract Momordica 7% extract, Licorice Root 20% extract</td>
</tr>
<tr>
<td>Gurmar powder</td>
<td>Garry and Sun natural</td>
<td>Gurmar (Gymnema sylvestre)</td>
</tr>
<tr>
<td>Remedies</td>
<td>Remedies</td>
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<td>----------------------------------------------</td>
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<tr>
<td>Epinsulin</td>
<td>Swastik</td>
<td></td>
</tr>
<tr>
<td>Formulations</td>
<td>vijaysar (Pterocarpus marsupium)</td>
<td></td>
</tr>
<tr>
<td>Diabecure</td>
<td>Nature beaute sante</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Juglans regia, Berberis vulgaris, Erytherea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>centaurium, Millefolium, Taraxacum</td>
<td></td>
</tr>
<tr>
<td>Diabeta</td>
<td>Ayurvedic cure Ayurvedic Herbal Health</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Products Gymnema sylvestre, Vinca rosea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Periwinkle), Curcuma longa (Turmeric),</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Azadirachta indica (Neem), Pterocarpus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>marsupium (Kino Tree), Momordica charantia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Bitter Gourd), Syzygium cumini (Black Plum),</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acacia arabica (Black Babhul), Tinospora</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cordifolia, Zingiber officinale (Ginger)</td>
<td></td>
</tr>
<tr>
<td>Syndrex</td>
<td>Plethico Laboretaries Germinated Fenugreek</td>
<td></td>
</tr>
<tr>
<td></td>
<td>seed extract</td>
<td></td>
</tr>
</tbody>
</table>

A number of plants have constituents which have antidiabetic properties when taken orally (Oliver-Bever., 1986). There is great diversity in the nature and action of these constituents, but a number of them do seem to belong to certain chemical classes, such as sitosterol glycosides (eg: β sitosterol 3-β-D-glucoside from the bark of Ficus religiosa), Alkaloids (eg: Galegin from the seeds of Galega officinalis L), sulphur compounds (eg: S-methyl cysteine sulfoxide from allium cepa), Flavonoids (eg: Epicatechin from the bark of Pterocarpus marsupium ROXb) and glycans or glycoproteins (eg: Ephedrans A,B,C,D and E from the aerial parts of Ephedra distachya L. Ephedraceae, and oryzabrans A,B,C and D from external seed coats of oryza sativa L. Graminae etc).

The glycosides isolated from the species belonging to the families of caesalpinaceae, compositae, convolvulaceae, Ericaceae, Moraceae, Mytaceae, Papavaraceae, Ranunculaceae Rhamnaceae and Scrophulaceae afforded active principle which lowered blood sugar in test animals. Similarly glycans and triterpenes of species of Ranunculaceae and glycans of Graminae exhibited similar activity (Oliver-Bever., 1986). In plants of Liliaceae this property was attributed to various types of sulfide molecules. Polysacharides, oils and vitamins from the family Graminae also showed pharmacological activity by decreasing blood sugar level in animals (Kameswarao et al., 1997). Alkaloids of Apocyanaceae, Papavernelaceae, Rhamnaceae and Zygophyllanceae were particularly effective in diabetes. Saponin from Araliaceae,
glycoproteins from Malvaceae, peptides, amino acids and proteins from papilionaceae and Rubiaceae families also showed beneficial effects in reducing the blood sugar (Oliver-Bever., 1986). Three alkaloids leurosine, Vindoline and Vindolinine which were isolated from *catharanthus roseus* showed good hypoglycemic activity. Trigonelline is a hypoglycemic principle isolated from *Trigonella foenumgraecum* (fenugreek) seeds. S–Methyl cysteine sulphoxide and S– allyl cysteine sulphoxide are antihyperglycemic agents isolated from the bulbs *Allium cepa* and *Allium sativum* respectively. Epicatechine from the bark of *Pterocarpus marsupium* showed insulin mimetic activity (Chakravarthy et al., 1981a; Chakravarthy et al., 1985). Hexane fraction of *swertia chirata* (swerchirin, 1,8–dihydroxy–3,5–dimethoxy Xanthone) is potent to reduce blood glucose levels (Saxena et al., 1991). Aqueous extract of tender leaves of neem (*Azadirachta indica*) neem oil, nimbin and acetyl nimbin and nimbolid from the leaves of *Azadirachta indica* shown antidiabetic activity. *M.Cy* protein is an antidiabetic active principle isolated from fruit of *Momordica cymbalaria* (Rajasekhar et al., 2010).

The Gymnemic acid isolated from the leaves of *Gymnema sylvestre*, which are chewed in India reduce glycosuria and normalize the blood sugar in diabetic patients in about 3- 4 weeks (Oliver-Bever., 1986 and Kameswarao et al., 1997). Patel et al., 2012 have listed some plants which have shown insulin secretagogue activity (*Table 4*). Earlier studies from our laboratory have reported the insulin secretagogue activities of *Momordica cymbalaria*, *Terminalia pallida* and *Syzygium alternifolium* in experimental diabetic animals (Kameswar roa et al., 2003; Sampath et al., 2008; Ramesh babu kasetti et al., 2010).

**Table 4: List of the plants having insulin mimetic or insulin secretagogue activity.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Plant botanical name</th>
<th>Common name</th>
<th>Family</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Abies pindrow</td>
<td>Morinda</td>
<td>Pinaceae</td>
<td>Insulin secretagogue activity</td>
</tr>
<tr>
<td>2.</td>
<td>Acacia arabica</td>
<td>Babool</td>
<td>Leguminosae</td>
<td>Release of insulin from pancrease</td>
</tr>
<tr>
<td>3.</td>
<td>Agrimony eupatoria</td>
<td>Rosaceae</td>
<td>Leaves</td>
<td>Insulin releasing and insulin like activity</td>
</tr>
<tr>
<td>4.</td>
<td>Aloe barbadensis</td>
<td>Gheequar</td>
<td>Liliaceae</td>
<td>Stimulating synthesis and release of insulin</td>
</tr>
<tr>
<td></td>
<td>Plant Name</td>
<td>Common Name</td>
<td>Family</td>
<td>Effect</td>
</tr>
<tr>
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<td>---------------------------------------------</td>
</tr>
<tr>
<td>5.</td>
<td><em>Annona squamosa</em></td>
<td>Sharifa</td>
<td>Annonaceae</td>
<td>Increased plasma insulin level</td>
</tr>
<tr>
<td>6.</td>
<td><em>Averrhoa bilimbi</em></td>
<td>Bilimbi</td>
<td>Oxalidaceae</td>
<td>Increase serum insulin level</td>
</tr>
<tr>
<td>7.</td>
<td><em>Bixa orellana</em></td>
<td>Annotta</td>
<td>Bixaceae</td>
<td>Increase plasma insulin concentration and increase insulin binding on insulin receptor</td>
</tr>
<tr>
<td>8.</td>
<td><em>Boerhaavia difusa</em></td>
<td>Punamava</td>
<td>Nyctaginaceae</td>
<td>Increase plasma insulin concentration</td>
</tr>
<tr>
<td>9.</td>
<td><em>Camellia sinensis</em></td>
<td>Green tea</td>
<td>Theaceae</td>
<td>Increase insulin secretion</td>
</tr>
<tr>
<td>10</td>
<td><em>Capsicum frutescens</em></td>
<td>Mirch</td>
<td>Solanaceae</td>
<td>Increase insulin secretion and reduction of insulin binding on the insulin receptor</td>
</tr>
<tr>
<td>11</td>
<td><em>Cinnamomum zeylanicum</em></td>
<td>Dalchini</td>
<td>Lauraceae</td>
<td>Elevation in plasma insulin level</td>
</tr>
<tr>
<td>12</td>
<td><em>Clausena anisata</em></td>
<td>Horse wood</td>
<td>Rutaceae</td>
<td>Stimulate secretion of insulin</td>
</tr>
<tr>
<td>13</td>
<td><em>Eucalyptus globulus</em></td>
<td>Eucalyptus</td>
<td>Myrtaceae</td>
<td>Increase insulin secretion from clonal pancreatic beta line (BRIN-BD 11)</td>
</tr>
<tr>
<td>14</td>
<td><em>Ficus religiosa</em></td>
<td>Peepal</td>
<td>Moraceae</td>
<td>Initiating release of insulin</td>
</tr>
<tr>
<td>15</td>
<td><em>Hibiscus rosa</em></td>
<td>Gudhal</td>
<td>Malvaceae</td>
<td>Stimulate insulin secretion from beta cells</td>
</tr>
<tr>
<td>16</td>
<td><em>Helicteres isora</em></td>
<td>Indian screw trees</td>
<td>Sterculiaceae</td>
<td>Decrease plasma triglyceride level and insulin sensitizing Activity</td>
</tr>
<tr>
<td>17</td>
<td><em>Ipomoea batata</em></td>
<td>Shakarkand</td>
<td>Convolvulaceae</td>
<td>Reduce insulin resistance and blood glucose level</td>
</tr>
<tr>
<td>18</td>
<td><em>Juniperus communis</em></td>
<td>Hauber</td>
<td>Pinaceae</td>
<td>Increase peripheral glucose consumption and</td>
</tr>
<tr>
<td></td>
<td>Species Name</td>
<td>Common Name</td>
<td>Family</td>
<td>Effect on Diabetes</td>
</tr>
<tr>
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</tr>
<tr>
<td>19.</td>
<td><em>Olea europia</em></td>
<td>Olive</td>
<td>Oleaceae</td>
<td>Increase insulin release and increase peripheral uptake of Glucose</td>
</tr>
<tr>
<td>20.</td>
<td><em>Swertia chirayata</em></td>
<td>Chirayata</td>
<td>Gentianaceae</td>
<td>Stimulates insulin release from islets</td>
</tr>
<tr>
<td>21.</td>
<td><em>Scoparia dulcis</em></td>
<td>Mithi patti</td>
<td>Scrophulariaceae</td>
<td>Insulin-secretagogue activity</td>
</tr>
<tr>
<td>22.</td>
<td><em>Tinospora crispa</em></td>
<td>Giloe</td>
<td>Menispermacae</td>
<td>Anti-hyperglycemic, stimulates insulin release from islets</td>
</tr>
<tr>
<td>23.</td>
<td><em>Urtica dioica</em></td>
<td>Bichhu booti</td>
<td>Urticaceae</td>
<td>Increase insulin secretion</td>
</tr>
<tr>
<td>24.</td>
<td><em>Vinca rosea</em></td>
<td>Sadabahar</td>
<td>Apocynaceae</td>
<td>Beta cell rejuvenation, regeneration and stimulation</td>
</tr>
<tr>
<td>25.</td>
<td><em>Zingiber officinale</em></td>
<td>Adrak</td>
<td>Zingiberaceae</td>
<td>Increase insulin level and decrease fasting glucose level</td>
</tr>
</tbody>
</table>

Due to the enormous costs of modern treatment for diabetes in developing countries, the use of medicinal plants and their preparation has flourished as an alternative for the control and prevention of the disease (Luo et al., 1998). Herbal remedies are apparently effective, produce minimal or no side effects in clinical experience and are of relatively low costs as compared to oral synthetic hypoglycemic agents (Gupta et al., 2005). Tirumala hills, which lie geographically in the South-Eastern Ghats, are known for their rich heritage of flora. A number of plants with known and unknown medicinal values are available here (Thammanna et al., 1990; 1994). The area is inhabited by a number of tribes which include Chenchus, Nakkalas, Sugalis, Yanadis and Yerukalas.

*Heliotropium indicum* is one of the medicinal plants used in the traditional medicine for the treatment of diabetes (Okvirk et al., 2013; Devi et al., 2011). The other species of this family *Heliotropium Zeylanicum* was reported to possess antidiabetic, antioxidant and antihyperlipidemic activities in STZ induced diabetic rats (Murugesh et al., 2006). But there are no systematic scientific studies on the antidiabetic activity of *Heliotropium indicum*. Hence the present study was undertaken to evaluate the antihyperglycemic activity of *Heliotropium indicum* in STZ induced diabetic rats with the following objectives.
OBJECTIVES

1. Checking the crude aqueous suspension of *Heliotropium indicum* (whole plant) for its antidiabetic activity.

2. Preparation and screening of different solvent extracts of *Heliotropium indicum* in STZ induced diabetic rats.

3. Phytochemical analysis of different solvent extracts of *Heliotropium indicum*.

4. On the basis of phytochemical analysis, Preparation of alkaloid rich fraction of *Heliotropium indicum* (ARFHI) and screening of different doses of ARFHI in normal and STZ induced diabetic rats.

5. Effect of ARFHI on Oral glucose tolerance.

6. To study the effect of long term treatment of diabetic rats with the alkaloid rich fraction of *Heliotropium indicum* (ARFHI) on,

   A. Glycemic control and associated changes in plasma insulin

   B. The activities of carbohydrate metabolizing enzymes and the levels of glycoprotein derivatives

   C. Serum lipids and lipoprotein profile

   D. Lipid peroxidation, enzymatic and non enzymatic antioxidant status

   E. Effect on hepatic and renal function markers

   F. Histopathological changes in different tissues of normal, diabetic untreated and treated rats.