Review of Literature
REVIEW OF LITERATURE

Diabetes mellitus is an endocrine disorder of carbohydrate metabolism resulting from inadequate insulin release (insulin dependent diabetes mellitus, or type 1 diabetes) or insulin insensitivity (non-insulin-dependent diabetes mellitus, or type 2 diabetes), both of which result in hyperglycemia if uncontrolled (Kumar 2002). It is mainly characterised by symptoms such as polydypsia, polyuria, blurring of vision and weight loss (Kumar 2002). Diabetes in absence of effective treatment can lead to diabetic ketoacidosis that may cause stupor, coma and even death. Diabetes has been found to be associated with a cohort of macrovascular and microvascular complications which is responsible for its devastating effects. Macrovascular complications mainly include cardiomyopathy, myopathy and stroke. Whereas, diabetes induced microvascular complications can lead to retinopathy, nephropathy, neuropathy as well as encephalopathy (Cade 2008). Diabetes can cause considerable functional and structural damage to CNS thereby causing significant cognitive deficits in human population (Dejgaard et al. 1991).

2.1 EPIDEMIOLOGY

The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8 % in 2000 and is projected to be 4.4 % in 2030 (Wild et al. 2004). The total number of people with diabetes is expected to rise from 171 million in 2000 to 366 million in 2030. International Diabetes Federation (2007) found that in the world’s 1.8 billion children were under age of 14. Prevalence of type 1 diabetes has been found to be 0.02 %, with about 70,000 new cases diagnosed annually and an average annual increment in incidence of 3 %. It has also been found that type 1 diabetes is the second most common chronic disease in children, accounting for 5 % to 10 % of all diagnosed cases of diabetes (Raha et al. 2009). The epidemiological study indicated that the prevalence of type 1 diabetes in India has also increased (Wadwa et al. 2005).

2.2 TYPES OF DIABETES

Two major classes of diabetes mellitus has been proposed by an expert committee of WHO and named as insulin dependent diabetes mellitus or Type 1 and non-insulin dependent diabetes mellitus or Type 2 (WHO 1999).

2.2.1 TYPE 1 DIABETES

Type 1 diabetes (insulin dependent diabetes mellitus) is characterised by a condition in which pancreatic β cell destruction occurs that causes absolute insulin deficiency
Two forms of type 1 diabetes have been identified that are: (i) Type 1A that results from a cell-mediated autoimmune attack on β-cells and (ii) Type 1B occurs mostly in individuals having varying degrees of insulin deficiency between sporadic episodes of ketoacidosis but is far less frequent and its cause is not known yet. Type 1 diabetes accounts for about 5–10% of all cases of diabetes; however, it has serious short-term and long-term implications.

2.2.1.1 EPIDEMIOLOGY OF TYPE 1 DIABETES

The incidence of type 1 disease is rapidly increasing in specific regions and is highly variable among different ethnic populations (LaPorte et al. 1985). The overall age-adjusted incidence of type 1 diabetes varies from 0.1/100,000 per year in China to more than 40/100,000 per year in Finland (Onkamo et al. 1999). The wide disparities in risk within ethnic groups can be due to differences in genes or environment. Furthermore, the incidence of type 1 diabetes seems to be increasing in almost all populations (Atkinson & Eisenbarth 2001). The incidence of type 1 diabetes was about 40% higher in 2010 than in 1997. Type 1 diabetes has also shown a trend towards earlier onset (Onkamo et al. 1999). Examination of the rates of type 1 diabetes as a function of the age at onset showed rates of increase of 6%, 3% and 2% in populations of children aged 0–4 years, 5–9 years and 10–14 years, respectively (Atkinson & Eisenbarth 2001). These findings support that more and more cases of type 1 diabetes, especially in younger children are appearing. This disorder has a strong genetic component, inherited mainly through the human leucocyte antigen (HLA) complex, but the factors that trigger onset of clinical disease remain largely unknown (Buzzetti et al. 1998).

2.2.1.2 PATHOGENESIS OF TYPE 1 DIABETES

An understanding of type 1 diabetes pathogenesis helps to determine its causes as well as to develop means to prevent and cure the disorder.

(A) Genetic factors

Type 1 diabetes represents a heterogeneous and polygenic disorder with a number of HLA and non-HLA loci contributing to disease susceptibility (Todd 1999). Genes for type 1 diabetes provide both susceptibility as well as protection from the disease. Susceptibility of type 1 diabetes is largely inherited depending on genes located within the major histocompatibility complex (MHC) HLA class II region on chromosome 6p21, termed (Buzzetti et al. 1998). The HLA locus is thought to confer about 45% of the genetic
susceptibility and roughly 15 % from other two non-HLA genes with minor contributions from the other genes (Redondo et al. 2001). These susceptibility genes are important regulators of the immune response i.e. presentation of antigenic peptides to T lymphocytes, protection associated with thymic insulin message, T-cell activation, thyroid autoimmunity yet their specific contribution to the pathogenesis of type 1 diabetes remains unclear (Todd 1999). The major risk factors for this disease involve association with HLA-DR3 and HLA-DR4 along with additional susceptibility related to DQ α chains and DQ β chains. Certain MHC haplotypes also provide significant protection, dominant over susceptibility in type 1 diabetes. Other genes associated with either rare syndromes including diabetes (eg, AIRE and Foxp3) or other autoimmune conditions (eg, PTPN22) might also provide important insights into the immune pathogenesis of type 1 diabetes (Daneman 2006).

(B) Environmental factors

Environmental agents may serve as modifiers of type 1 disease pathogenesis. Environmental risk determinants for type 1 disease have been classified into three groups: viral infections (eg, coxsackievirus and cytomegalovirus), early infant diet (eg, breast feeding versus early introduction of cow’s milk components) and toxins (eg, N-nitroso derivatives) (Ellis & Atkinson 1996, Dahlquist 1997). Exposure to one or more environmental factors may alter immune function, thereby initiating cell destruction. Non-genetic disease-modifying factors mainly include vaccine administration, psychological stress and climatic influences. Life-long influence of multiple environmental factors such as infectious agents, dietary factors and environmental toxins affects penetrance as well as expression of heritable immune aberrations in this disease (Knip & Akerblom 1999). The dramatically increased frequency of type 1 diabetes during the past three decades can be attributed to changing environmental factors.

(C) Islet cell autoantibodies

The abnormal activation of the T-cell-mediated immune system in susceptible individuals leads to an inflammatory response within the islets (insulitis) as well as humoral (B cell) response with production of antibodies to β-cell antigens. Islet cell autoantibodies mainly are against insulin (IAA), glutamic acid decarboxylase (GADA/GAA) and the protein tyrosine phosphatase IA2 (IA-2AA), responsible for autoimmune destruction of islet cells (Devendra et al. 2004). The presence and persistence of positivity to multiple antibodies increases the likelihood of progression to this disease (Krischer et al. 2003).
β-cells destruction is a diverse as well as complex mechanism and is poorly understood. This autoimmune disorder involves both innate and acquired immune responses (Yoon & Jun 2005). Genetically susceptible individuals have autoreactivity against several autoantigens such as GAD, IAA, IA2 etc. The immune response to autoantigens is likely to be initiated by antigen presenting cells (APCs) (van Belle et al. 2011). Exogenous antigen binds to HLA class II molecules, resulting in an antigenic shift to an autoantigen. Misrecognition of the trimolecular complex of HLA and peptide may stimulate helper T cells that assist B lymphocytes to make autoantibodies which react with an autoantigen. This presentation of an antigen is carried out by an APC such as macrophage and dendritic cell. All these events lead to production of cascade of cytokines like interleukin-12 (IL-12), interferon-γ (IFN-γ), interleukin 4 (IL-4), interleukin 10 (IL-10) etc thereby activating cell mediated and humoral responses, thereby destroying β-cells as shown in Fig. 1.

Figure 1: Etiology of type 1 diabetes. Autoantigens released from β cells are processed by antigen-presenting cells (APCs) and presented to helper T cells (Th cells) associated with MHC class II molecules. IL-12 released from APCs activates CD4+ T cells, causing breakdown of effector and regulatory cells. TH1 cells produce IL-2, which activates β cell-precytotoxic T cells (Pre CTL) to become CTL and IFN-γ, which make macrophages cytotoxic. These cytotoxic macrophages and TH1 cells release cytokines IL-1β, TNF-α, IFN-γ and free radicals. β Cellspecific CD8+ CTL recognize antigens expressed on β cells in association with MHC class I molecules. These CTLs release granzyme and perforin (cytolysin), which are toxic to β cells. In addition, Fas- and TNFR-mediated apoptosis are involved in β cell destruction (Yoon & Jun 2005).
Increasing β cells destruction leads to progressive loss of insulin secretory reserve leading to clinical diabetes where insulin secretion falls below a critical amount and onset of type 1 diabetes occurs (Foulis et al. 1986). Firstly, high rate of β cell apoptosis takes place due to intrinsic insulin resistance that comes into play due to weight gain and physical inactivity. Insulin resistance puts pressure on β cell mass (risk for accelerated apoptosis) contributing to the expression of clinical diabetes. Then absolute insulin deficiency takes place due to genetically determined predisposition to β cell autoimmunity. At young age, β cells are destroyed more rapidly after onset of clinical diabetes. Type 1 diabetic individuals are also more susceptible to other autoimmune conditions including Hashimoto’s thyroiditis, Addison’s disease, celiac disease, myasthenia gravis and vitiligo (Barker et al. 2005).

### 2.2.1.3 DIAGNOSIS OF TYPE 1 DIABETES

The primary diagnosis of diabetes is made on the basis of hyperglycaemia (by fasting plasma glucose levels as described in **Table 1**) and symptoms like nausea, vomiting, acute illness, polyuria, polydypsia and weight loss. Oral glucose tolerance test (OGTT) and glycosylated haemoglobin (HbA1c) are more specific tests than fasting plasma glucose levels, also used for diabetes diagnosis nowadays. However, anti-islet autoantibody determination with specific assays (eg, IAA, GADA), can provide a specific and precise tool to diagnose type 1A diabetes. About 90% of children have at least one of these autoantibodies at disease diagnosis. 5–30% of adults with what initially seems to be type 2 diabetes (including overweight non-insulin-treated adults) has anti-islet autoantibodies and in fact has a variant of type 1A diabetes (Daneman 2006).

#### Table 1: Glycaemia and HbA1c for adults in type 1 diabetes (Daneman 2006).

<table>
<thead>
<tr>
<th></th>
<th>HbA1c (%)</th>
<th>FPG/preprandial PG (mmol/L)</th>
<th>2h postprandial PG (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target for most patients</td>
<td>&lt;7.0</td>
<td>4.0-7.0</td>
<td>5.0-11.0</td>
</tr>
<tr>
<td>Normal range</td>
<td>&lt;6.0</td>
<td>4.0-6.0</td>
<td>5.0-8.0</td>
</tr>
</tbody>
</table>

Treatment targets must be allowed must be tailored to the patient, with consideration given to individual risk factors. Normal range should be considered for patients in whom it can be achieved safely. HbA1C= haemoglobin A1c; FPG=fasting plasma glucose; PG= plasma glucose.
2.2.2 TYPE 2 DIABETES

Type 2 diabetes (non-insulin dependent diabetes mellitus) is characterized by two defects: insulin deficiency and insulin resistance. In this condition, pancreas functions normally and replenishes sufficient insulin to the blood but due to insulin resistance, cells are not able to use it thereby creating insulin deficiency. Type 2 diabetes accounts for 90 to 95% of the incidence of diabetes. The current epidemic outbreak of diabetes reflects the high prevalence of type 2 diabetes (Cheng 2005).

2.2.1.4 EPIDEMIOLOGY OF TYPE 2 DIABETES

Diabetes mellitus affects more than 170 million individuals worldwide and has become an epidemic (Stumvoll et al. 2005). It has been estimated that there was a growth of 50% in year 2010 mainly in the developing countries of Africa, Asia and South America. Prevalence of diabetes mellitus had reached about 6% in developed countries and even more alarmingly, 4% of adolescents had diabetes and 25% had abnormal glucose tolerance. 90% of diabetic individuals have type 2 diabetes mellitus (Zimmet et al. 2001). Type 2 diabetes has a multifactorial pathogenesis caused by alterations in several gene products and lifestyle.

2.2.1.5 DIAGNOSIS OF TYPE 2 DIABETES

Diabetes mellitus is diagnosed on the basis of both fasting and 2-h after glucose load (75 g) criteria (Table 2). About 7% of people with these symptoms progress to overt diabetes every year before being diagnosed. Furthermore, impaired glucose tolerance itself carries an increased risk of macrovascular disease.

Table 2: Diagnostic criteria for Type 2 diabetes (Stumvoll et al. 2005).

<table>
<thead>
<tr>
<th></th>
<th>Glucose concentration in venous plasma (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>Fasting &gt;7.0 or 2-h post glucose load &gt;11.1</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>Fasting &lt;7.0 and 2-h post glucose load &gt;7.8 and &lt;11.1</td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
<td>Fasting &gt;6.1 and &lt;7.0 and 2-h post glucose load &lt;7.8</td>
</tr>
</tbody>
</table>

Glucose load=75g glucose orally.


2.2.1.6 PATHOPHYSIOLOGY OF TYPE 2 DIABETES

Insulin is the key hormone for regulation of blood glucose and its homeostasis. In normal pancreatic β cells a decrease in insulin action is accompanied by upregulation of insulin secretion (and vice versa). β cell dysfunction is a critical component in the pathogenesis of type 2 diabetes. Whenever insulin action decreases, the system compensates it by increasing β-cell function. However at the same time, concentrations of blood glucose increase mildly leading to glucose toxicity causing β-cell dysfunction. Thus, even with unlimited β cell reserve, insulin resistance paves the way for hyperglycaemia and type 2 diabetes (Bergman 1989).

(A) Genetic factors

Many known and unknown genetic elements are involved in the pathogenesis of type 2 diabetes. Positive family history confers a nearly 2 fold higher risk for type 2 diabetes. The disease has a strong genetic component, but only a handful of genes have been identified so far: genes for calpain 10, potassium inward-rectifier 6·2, peroxisome proliferator-activated receptor γ, insulin receptor substrate-1 and others (McCarthy & Menzel 2001).

(B) Insulin resistance

Insulin resistance is a situation when biological effects of insulin are less than expected for both glucose disposal in skeletal muscle and suppression of endogenous glucose production primarily in the liver (Dinneen et al. 1992). Endogenous glucose production is increased in patients with type 2 diabetes. Increase in glucose production occurs in the presence of hyperinsulinaemia where hepatic insulin resistance is the driving force of hyperglycaemia of type 2 diabetes (Weyer et al. 1999).

(C) Obesity

Insulin resistance is strongly associated with obesity and physical inactivity. A number of hormones, cytokines and free fatty acids originate in the adipocyte and modulate insulin action (Boden 1997). Excessive fat storage in adipose cells and non adipose cells leads to increased release of free fatty acids and glycerol, both of which aggravate insulin resistance in skeletal muscle and liver (Fig. 2).

(D) Cellular mechanism

Insulin elicits its pleiotropic metabolic response by binding to and activating a specific plasma membrane receptor with tyrosine kinase activity followed by a cascade of
intracellular events depicted in Fig. 3. Negative modulation of insulin action can be mediated via various pathways leading to insulin resistance (White 2002).

![Pathophysiology of hyperglycaemia in type 2 diabetes.](image)

**Figure 2: Pathophysiology of hyperglycaemia in type 2 diabetes.** The various factors shown that contribute to the pathogenesis of type 2 diabetes affect both insulin secretion and insulin action which reduce insulin signalling in its target tissues. This leads to increased circulating fatty acids and the hyperglycaemia of diabetes. In turn, the raised concentrations of glucose and fatty acids in the bloodstream will feed back to worsen both insulin secretion and insulin resistance (Stumvoll et al. 2005).

*(E) β cell dysfunction*

Hyperinsulinemia occurs in type 2 diabetic due to increased plasma glucose concentrations as well as due to marked diminution of insulin secretion. Glucose is rapidly taken up by the pancreatic β cells via the glucose transporter 2 (GLUT-2) and converted to ATP. ATP leads to activation of the sulphonylurea receptor 1 (SUR1) protein which aids in opening of calcium channels, which triggers the release of preformed insulin-containing granules therefore hyperinsulinemia is preceded by hyperglycemia in type 2 diabetic individuals (Olokoba et al. 2012). Further in β cells, large amounts of ROS are formed due to hyperglycemia causing subsequent damage to cellular components and β cell loss. Thus, in type 2 diabetics an insulin secretory defect is present, possibly on a genetic basis. Obesity, acute illness or ageing further aggravates the underlying defect, ultimately leading to overt diabetes.
Figure 3: **Insulin signalling and insulin resistance.** Insulin signalling involves binding of insulin to its receptor followed by a cascade of intracellular events. Negative modulation of insulin action can be mediated via various pathways leading to insulin resistance and various inhibitory triggers affect their respective signal modulators (partly via transcription factors), which lead through deactivating pathways (tyrosine phosphatase, serine kinase, lipid phosphatase and degradation pathways) to inhibitory actions on insulin signalling (activation pathways). Adiponectin has an ameliorating function on glucose metabolism apart from insulin signalling. PKC-protein kinase C, PTEN phosphatase and tensin homologue. PI- phospho-inositol.

### 2.3 ANIMAL MODELS OF TYPE 1 DIABETES

Type 1 diabetes is characterised by autoimmune destruction of pancreatic β cells leading to deficiency of insulin production. In animal models, this lack of insulin production is achieved by a variety of different mechanisms, ranging from chemical ablation of the cells to breeding rodents that spontaneously develop autoimmune diabetes (Rees & Alcolado 2005). Some of the most commonly used models of type 1 diabetes are mentioned below.

#### 2.3.1 CHEMICALLY INDUCED TYPE 1 DIABETES MODEL

In chemically induced models of type 1 diabetes, a high percentage of the endogenous β cells are destroyed thereby causing a deficiency in endogenous insulin production leading to hyperglycaemia and weight loss. Chemically induced type 1 diabetes provides a simple and relatively cheap model of diabetes in rodents but can also be reproduced in higher animals (Dufrane et al. 2006). Diabetes is usually induced few days prior to the start of the experiment to ensure stable hyperglycaemia. Two main compounds that are used to induce
diabetes: streptozotocin (STZ) or alloxan (structural analogs of glucose). One disadvantage with chemically induced diabetes is that the chemicals can be toxic at other organs of the body as they tend to cause changes in P450 isozymes in the liver, kidney, lung, intestines, testis and brain (Bono 1976).

2.3.1.1 STREPTOZOTOCIN[2-deoxy-2-(3-(methyl-3-nitrosoureido)Dglucopyranose]

It is synthesized by *Streptomyces achromogenes* and is administered intraperitoneally/intravenously. It enters the pancreatic β cell through the GLUT-2 transporter due to similarity in structure with glucose and causes alkylation of the DNA (Szkudelski 2001). This event is followed by activation of PARP causing NAD+ depletion, a reduction in cellular ATP and subsequent inhibition of insulin production (Sandler & Swenne 1983). STZ is also a source of free radicals that contribute to DNA damage and subsequent cell death. Development of diabetes is dependent on cytokine production but it also develops even in the absence of T and B cells and therefore, it does not model the human disease as closely as spontaneous models of autoimmunity (Atkinson & Leiter 1999).

2.3.1.2 ALLOXAN (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil)

Alloxan is readily absorbed by the β cells thereby forming free radicals in the absence of proper defense mechanisms causing their destruction and creating a diabetic state (Nerup et al. 1994). Alloxan is reduced to dialuric acid and then re-oxidized back to alloxan, creating a redox cycle causing generation of superoxide radicals that undergo dismutation to form hydrogen peroxide and thereafter highly reactive hydroxyl radicals that cause fragmentation of DNA (Szkudelski 2001). Alloxan is also taken up by the liver, but it has better defense mechanism and therefore is not as susceptible to damage (Malaisse et al. 1982, Mathews & Leiter 1999). Other mechanisms of β cell damage by alloxan include oxidation of essential thiol groups, especially that of glucokinase (im Walde et al. 2002) and disturbances in intracellular calcium homeostasis (Kim et al. 1994).

2.3.2 SPONTANEOUS AUTOIMMUNE MODELS OF TYPE 1 DIABETES

The most commonly used autoimmune models of type 1 diabetes are the non-obese diabetic (NOD) mouse and the Biobreeding (BB) rat (Yang & Santamaria 2006). In addition, another rat model of autoimmune type 1 diabetes is LEW.1AR1/Ztm-iddm rat has also been described (Lenzen et al. 2001).
2.3.2.1 NOD MICE

NOD mice develop insulitis at around 3–4 weeks of age and was developed at the Shionogi Research Laboratories in Osaka, Japan in 1974 (Hanafusa et al. 1987). In this model, the pancreatic islets are infiltrated by predominately CD4+ and CD8+ lymphocytes, although B cells and NK cells are also present (Yoon & Jun 2001). The insulitis causes β cell destruction, but the onset of overt diabetes is apparent at around 10–14 weeks (Pozzilli et al. 1993). NOD mice share structural similarities to that in humans in terms of MHC class II, which may confer resistance or susceptibility to the disease in both NOD mice and humans therefore it become extremely useful in dissecting some mechanisms and pathways behind type 1 diabetes (Driver et al. 2011, Yang & Santamaria 2006). NOD mouse has helped in identifying many of the genetic and signalling pathways that can lead to type 1 diabetes.

2.3.2.2 BB RATS

BB rats, derived from outbred Wistar rats have been used to develop spontaneous autoimmune diabetes (Mordes et al. 2004). BB rats develop diabetes just after puberty (between 8 and 16 weeks) and diabetic phenotype is quite severe. In this model, animals have insulitis with the presence of T cells, B cells, macrophages and NK cells. The main disadvantage of BB model is the formation of lymphopenia which is not a characteristic of type 1 diabetes in humans. However, the model has been valuable in elucidating more about the genetics and complications of type 1 diabetes (Wallis et al. 2009).

2.3.2.3 LEW.1AR1/iddm rats

This rat model of type 1 diabetes is a spontaneous occurring model in congenic Lewis rats with a defined MHC haplotype (LEW.1AR1). These rats exhibit insulitis and overt diabetes manifests at around 8–9 weeks with an incidence rate of 60% (Jorns et al. 2014). The animals exhibit a prediabetic period with islet infiltration that helps in effective analysis of different stages of the immune cell infiltration (Weiss et al. 2005). They can be used to study diabetic complications (Jorns et al. 2014) as well as mechanisms involved in the development of diabetes and intervention studies (Peschke et al. 2011).

2.3.3 GENETICALLY INDUCED INSULIN-DEPENDENT DIABETES

2.3.3.1 AKITA MICE

The AKITA mouse has a spontaneous mutation in the insulin 2 gene preventing correct processing of proinsulin. This causes an overload of misfolded proteins and
subsequent endoplasmic reticulum (ER) stress resulting in severe insulin dependent diabetes. This model is commonly used to study ER stress in the islets (Chen et al. 2011).

### 2.3.3.2 VIRUS INDUCED MODELS OF DIABETES

Several animal models have used viruses to initiate β cell destruction as they play an important role in pathogenesis of type 1 diabetes. The destruction can be either due to infection of the β cell or initiation of an autoimmune response (Yoon & Jun 2001). Viruses used to induce diabetes in animal models include coxsackie B virus (Jaidane et al. 2009), encephalomyocarditis virus (Shimada & Maruyama 2004) and Kilham rat virus (Guberski et al. 1991). In addition, a transgenic virus model has been described in which a defined viral antigen of lymphocytic choriomeningitis virus (LCMV) is expressed under the rat insulin promoter which when injected with LCMV, causes β cell destruction (von Herrath et al. 2002).

### Table 3: Rodent models of Type 1 diabetes mellitus

<table>
<thead>
<tr>
<th>Induction of Mechanism</th>
<th>Model</th>
<th>Main features</th>
<th>Possible uses</th>
</tr>
</thead>
</table>
| Chemically induced     | Streptozotocin Alloxan | • Simple model of hyperglycemia  
• Model of induced insulitis | • New formulations of insulin  
• Treatments that may prevent β cell death  
• To study complications of diabetes |
| Spontaneous autoimmune | NOD mice  
BB rats  
LEW.1AR1/iddm rats | • Structural similarities to that in humans  
• β cell death due to an autoimmune process | • Understanding genetics of TIDM  
• Understanding mechanism and signalling of TIDM |
| Genetically induced    | AKITA mice | • β cell death due to ER stress | • New formulations of insulin  
Treatment to prevent ER stress |
| Virus-induced          | Coxsackie B virus  
Encephalomyocarditis virus  
Kilham rat virus | • β cell death induced by viral infection of beta cells | • Establish potential role of viruses in development of TIDM |

### 2.4 COMPLICATIONS OF DIABETES MELLITUS

Long-term diabetes-related complications are further classified into microvascular and macrovascular disorders, which account for most of the increased morbidity and mortality associated with the disease (Nathan 1993).
2.4.1 MACROVASCULAR COMPILICATIONS

Diabetes induced macrovascular complications mainly comprises of complications that affect large vessels (> 100µm) and they include cardiovascular diseases like myocardial infarction, stroke, ischemic heart disease, large vessel peripheral vascular disorder, cerebrovascular disease. Cardiovascular disease (CVD) is the primary cause of death in people with either type 1 or type 2 diabetes.

2.4.1.1 CARDIOVASCULAR DISEASE

Type 2 diabetes is mainly characterised by factors like abdominal obesity, hypertension, hyperlipidemia and increased coagulability that are also the risk factors for CVD (Gillum 1987). Type 2 diabetes independently acts as a risk factor for the development of ischemic disease, stroke and death. Type 1 diabetes also bears a burden of coronary heart disease mainly ischemic heart disease. Diabetes increases the risk of developing CVD as it increases the likelihood of atherosclerotic plaque formation (Kannel & McGee 1979). The central pathological mechanism in macrovascular disease is the process of atherosclerosis that leads to narrowing of arterial walls throughout the body.

2.4.1.2 Atherosclerosis

Atherosclerosis is a result of chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system. In diabetic arteries, endothelial dysfunction involve insulin resistance specific to the phosphatidylinositol-3 (PI-3) kinase pathway and hyperglycaemia (Beckman et al. 2002). Insulin resistance and hyperglycemia results in decreased endothelial production of the anti-atherogenic molecule nitric oxide, increased potentiation of proliferation of vascular smooth muscle cells and production of plasminogen activator inhibitor-1 (PAI-1) via the Ras → Raf → MEK kinase → mitogen-activated protein (MAP) kinase pathway. Oxidized lipids from low density lipoproteins (LDL) particles also accumulate in the endothelial wall of arteries in response to endothelial injury and inflammation. Monocytes then infiltrate the arterial wall and differentiate into macrophages, which accumulate oxidized lipids to form foam cells. Foam cells then stimulate macrophage proliferation and attract T-lymphocytes. Then, T-lymphocytes induce smooth muscle proliferation in the arterial walls and collagen accumulation. The result of this process is the formation of a lipid rich atherosclerotic lesion with a fibrous cap and its rupture leads to acute vascular infarction. Atheroma formation, increased platelet adhesion and hypercoagulability also occur in type 2 diabetes. Impaired NO generation, increased free radical formation and
altered calcium regulation, promote platelet aggregation and impair fibrinolysis in type 2 diabetic patients and further increases the risk of vascular occlusion and cardiovascular events in type 2 diabetes (Colwell et al. 1983).

2.4.1.3 HYPERTENSION

CVDs are also supported by factors like hypertension, contributing to its high prevalence (Kojda & Harrison 1999). It has been suggested that hypertensive persons are more predisposed to the development of diabetes than normotensive persons. 75% of CVDs in diabetes are attributable to hypertension.

2.4.1.4 DIABETIC CARDIOMYOPATHY

Diabetic cardiomyopathy is a diabetes-related myopathic state mainly characterized by impaired diastolic function (Hamby et al. 1974). Diabetes leads to altered mechanical properties of the myocardium and cardiomyocytes causing prolongation of contraction and relaxation as well as slowing in relaxation velocity (Aneja et al. 2008). Diabetic cardiomyopathic state is due to altered K+ channel function, alterations in Na+ pump function, alterations in sarcoplasmic reticulum Ca2+-ATPase, Na+-Ca2+exchanger function and abnormalities of PKC metabolism. It is also associated with a balance between the cardiac Ras and insulin-like growth factor (IGF)-1 (Sowers et al. 2001). Overexpression of Ras in the diabetic heart predispose resistance to the actions of insulin/IGF-1 on the PI3-kinase–mediated activation of K+ channel and Na+ pump expression/activation as well as myofilament-Ca2+ sensitivity. These abnormalities causes decreased expression/activation of the K+ channel and Na+ pump in both type 1 and type 2 diabetic states thereby disrupting diastolic and systolic function and left ventricular hypertrophy, which characterize “diabetic cardiomyopathy.”

2.4.1.5 PERIPHERAL VASCULAR DISEASE

Peripheral vascular disease (PVD), like the other mentioned vascular diseases, is related to the duration and severity of diabetes (Jude et al. 2001). Hyperglycemia, specifically, glycated hemoglobin, has been shown to be an independent risk factor for PVD (Selvin et al. 2004). People with diabetes are 15 times more likely to have lower-extremity amputation than people without diabetes (Dickinson et al. 2002). PVD is characterized by occlusion of the lower-extremity arteries, which can cause intermittent claudication and pain, especially upon exercise and activity resulting in functional impairments and disability (McDermott et al. 2004). Atheromatous disease in the legs, as in the heart, tends to affect
more distal vessels like tibial arteries producing multiple, diffuse lesions. In addition to diabetes, other risk factors for PVD include hypertension, tobacco use, obesity (ie, waist-to-hip ratio), elevated serum fibrinogen levels, dyslipidemia, history of CVD and physical inactivity (Wattanakit et al. 2005).

2.4.1.6 CEREBROVASCULAR DISEASE

Diabetes has been found to be an independent risk factor of stroke and cerebrovascular disease, as in coronary artery disease, the risk in people with diabetes is up to 2 to 4 fold greater (Lehto et al. 1996). Patients with diabetes are probably more prone to irreversible rather than reversible ischemic brain damage associated with small lacunar infarcts. Diabetic people have a stroke with more severe neurological deficits and a poorer long-term prognosis. Risk of stroke related dementia and recurrence as well as stroke related mortality, is elevated in patients with diabetes (Elneihoum et al. 1998). As in CVD, the presence of diabetes adversely affects the cerebrovascular circulation by increasing the risk of intracranial and extracranial (eg, carotid artery) atherosclerosis (Fabris et al. 1994). Hyperglycemia is a significant predictor of fatal and nonfatal stroke. People with diabetes have an increased incidence of various risk factors for stroke, including hypertension, dyslipidemia, heart failure and atrial fibrillation. Maintaining good glycemic control immediately after a stroke is likely to improve outcome, but the long term survival is reduced because of a high rate of recurrence (Stegmayr and Asplund 1995).

2.4.2 MICROVASCULAR COMPLICATIONS

Microvascular complications of diabetes mainly encompass long-term complications of diabetes affecting small blood vessels (< 100µm) causing diabetic nephropathy, neuropathy, retinopathy and encephalopathy (Fowler 2008).

2.4.2.1 DIABETIC RETINOPATHY

Diabetic retinopathy is the most common microvascular complication of diabetes (Davis 1992). The risk of developing diabetic retinopathy and diabetes related microvascular complications depends on duration and the severity of hyperglycemia (Brownlee 2005). Several pathological mechanisms have been proposed by which diabetes causes retinopathy. (i) High glucose levels increase the flux of sugar molecules through the polyol pathway, which causes sorbitol accumulation in cells (Lorenzi 2007). Polyol pathway involves the conversion of glucose into glucose alcohol (sorbitol). Sorbitol accumulation causes osmotic stress thereby developing diabetic microvascular complications including diabetic
Sorbitol accumulation has been linked to microaneurysm formation, thickening of basement membranes and loss of pericytes. (ii) Hyperglycemia also promotes the nonenzymatic formation of AGEs (Zong et al. 2011). They are also associated with formation of microaneurysms and pericyte loss. (iii) Oxidative stress also plays a key role in cellular injury from hyperglycemia (Wu et al. 2014). High glucose levels stimulate free radical production and ROS formation causing vascular dysfunction associated with diabetes. (iv) Growth factors like vascular endothelial growth factor (VEGF), growth hormone and transforming growth factor β (TGF-β) have been involved in the development of diabetic retinopathy (Gupta et al. 2013).

Diabetic retinopathy has features like small hemorrhages in the middle layers of the retina, hard exudates caused by lipid deposition at the margins of hemorrhages, microaneurysms (small vascular dilatations) that occur in the retina, retinal edema (Tarr et al. 2013). Retinal edema is associated with visual deterioration (Ferris & Patz 1984). Retinopathy is also characterized by the formation of new blood vessels on the surface of the retina and can lead to vitreous hemorrhage thereby causing blindness (Crawford et al. 2009). Therefore, surveillance for the progression of retinopathy in diabetics is crucial.

2.4.2.2 DIABETIC NEPHROPATHY

Diabetic nephropathy is the major cause of renal failure in many countries. It is defined by proteinuria > 500 mg in 24 hs at the onset of diabetes, but this is preceded by lower degrees of proteinuria or microalbuminuria which is defined as albumin excretion of 30–299 mg/24 hs (Maezawa et al. 2015). Diabetic patients with microalbuminuria progress to proteinuria and overt diabetic nephropathy (Currie et al. 2014). This progression occurs in both type 1 and type 2 diabetes. 7% of patients with type 2 diabetes already have microalbuminuria at the time they are diagnosed with diabetes. Studies suggest that cumulative incidence of microalbuminuria in patients with type 1 diabetes was ~ 12% during a period of 7 years (Hovind et al. 2004). Diabetic nephropathy is characterised by pathological changes that mainly include increased glomerular basement membrane thickness, microaneurysm formation, mesangial nodule formation (Kimmelsteil-Wilson bodies) and other changes (Araki 2014). The underlying mechanism of injury also involves some or all of the same mechanisms as diabetic retinopathy. Screening for diabetic nephropathy is accomplished by 24-hs urine collection or a spot urine measurement of microalbumin or by measuring microalbumin-to-creatinine ratio (Currie et al. 2014). There is a strong association between glucose control and the risk of developing diabetic nephropathy.
2.4.2.3 DIABETIC NEUROPATHY

Diabetic neuropathy is recognized by the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes (Boulton et al. 2005). Risk of developing diabetic neuropathy is proportional to both the magnitude as well as duration of hyperglycemia and some individuals may possess genetic attributes that affect their predisposition to developing such complications (Freedman et al. 2007). The precise mechanism of damage to the peripheral nerves from hyperglycemia is not known but it is likely to be related to mechanisms such as polyol accumulation, injury from AGEs and oxidative stress (Johnson et al. 1986). Peripheral neuropathy in diabetes can be manifested into several different forms, including sensory, focal/multifocal and autonomic neuropathies (Boulton & Malik 1998). Diabetic neuropathy leads to foot ulceration or injury resulting in 80% of amputations. Due to considerable morbidity and mortality that can result from diabetic neuropathy, it is important to understand its manifestations, prevention and treatment (Low et al. 2004). (i) Chronic sensorimotor distal symmetric polyneuropathy is the most common form of neuropathy in diabetes in which patients experience burning, tingling, loss of ankle reflex and “electrical” pain, or simple numbness. Symptoms are typically most prominent at night. Patients who have lost some monofilament sensation are at considerably elevated risk for developing foot ulceration (Selvarajah et al. 2008). (ii) Pure sensory neuropathy is relatively rare and associated with periods of poor glycemic control or considerable fluctuation in diabetes control. It is characterized by isolated sensory findings without signs of motor neuropathy (Thomas 1997). (iii) Mononeuropathies have a more sudden onset and involve virtually any nerve, but most commonly the median, ulnar and radial nerves are affected (Hawley 1996). (iv) Cranial neuropathies have also been seen in diabetics but incidence is rare. It should be noted that nerve entrapment occurs frequently in the setting of diabetes. Decrease in both amplitude of nerve impulse and conduction has been demonstrated by electrophysiological evaluation in diabetic neuropathy that may be useful in identifying the location of nerve entrapment (Thomas 1997). (v) Diabetic amyotrophy is a manifestation of diabetic mononeuropathy and is characterized by severe pain and muscle weakness and atrophy, usually in large thigh muscles. Several other forms of neuropathy may mimic the findings in diabetic sensory neuropathy and mononeuropathy (Kelkar et al. 2000).

Diabetic autonomic neuropathy causes significant morbidity and even mortality in patients with diabetes (Vinik & Erbas 2013). Neurological dysfunction may occur in most organ systems and can be manifested by gastroparesis, constipation, diarrhea, anhidrosis,
bladder dysfunction, erectile dysfunction, exercise intolerance, resting tachycardia, silent ischemia and even sudden cardiac death.

2.4.2.4 DIABETIC ENTEROPATHY

Diabetes mellitus has been found to be associated with various organ system dysfunctions including gastrointestinal involvement causing esophageal dysmotility, gastro-esophageal reflux disease, gastroparesis, enteropathy, non alcoholic fatty liver disease and glycogenic hepatopathy (Tsimmerman Ia & Zinnatullin 2011). Diabetic gastroparesis causes symptoms like early satiety, bloating, vomiting, abdominal pain and erratic glycemic control whereas diabetic enteropathy is also common and management involves glycemic control and symptomatic measures (Camilleri et al. 2013).

Small intestinal and colorectal dysfunctions have been found in patients with long standing diabetes (Camilleri 2007). Diabetic enteropathy may present with diarrhea, constipation or fecal incontinence. The mechanism of development of enteropathy in diabetes involves role of AGEs that cause damage to cellular DNA and tissues (Krishnan et al. 2013). AGEs and their receptors have been found to be increased in the ganglia, crypt and brush border of diabetic jejunum and ileum as well as in the ganglia of diabetic colon in animal models. Long standing diabetes results in damage to the myenteric nerve plexus due to autonomic neuropathy and fibrosis of the intestinal muscular layers causing stasis of the intestinal contents (Takahashi et al. 1997). Reduced bowel motility also results in constipation that may sometimes lead to overflow incontinence. Intestinal stasis also causes small intestinal bacterial overgrowth thereby resulting in diarrhea. Diabetic enteropathy also causes symptoms like constipation alternating with diarrhea (Spangeus et al. 1999). The diarrhea is typically painless and is associated with fecal incontinence occurring during the day but more often at night. This phenomenon has been seen more oftenly in patients with poorly controlled diabetes that has peripheral and autonomic neuropathy. Other causes of diarrhea in diabetics include pancreatic insufficiency, bile salt malabsorption, steatorrhea and drugs (Metformin) (Valdovinos et al. 1993).

Constipation is a common problem affecting up to 60 % of patients with long-standing diabetes mellitus (Feldman & Schiller 1983). Severe constipation leading to megacolon or colonic intestinal pseudo-obstruction occurs rarely while stercoral ulcer, perforation and overflow diarrhea are encountered infrequently. Due to internal and external sphincter dysfunction secondary to autonomic neuropathy resulting in fecal incontinence is a troublesome symptom particularly occurring nocturnally (Krishnan et al. 2013). Acute
hyperglycemia increases the risk of fecal incontinence by inhibiting external anal sphincter function and decreased rectal compliance. Treatment of diabetic diarrhea mainly involves symptom relief, correction of fluid and electrolyte deficits, improvement of nutrition and glycemic control and management of underlying causes (Shakil et al. 2008).

2.5 DIABETIC ENCEPHALOPATHY

Long term complications of diabetes affecting CNS are termed as diabetic encephalopathy (Mooradian 1988). Chronic diabetes is widely known to affect the functioning of the CNS. Diabetes induces CNS complications that may include structural alterations or brain atrophy, as well as changes in electrophysiological properties that ultimately result in deficits in cognitive performance (Gispen & Biessels 2000). These diabetes-induced CNS complications may be associated with or exacerbated by cardiovascular disease, including hypertension (Elias et al. 2005, Peila et al. 2002) and cerebral vascular complications (Tuttolomondo et al. 2008). Additional factors that may contribute to diabetes-induced cognitive impairment include disrupted insulin signalling and glucose homeostasis in the CNS (McNay & Recknagel 2011). Diabetes also leads to end organ damage in the CNS due to both acute and chronic metabolic and vascular disturbances causing hypoglycaemia and stroke (Klein & Waxman 2003).

Under normal circumstances glucose is the predominant metabolic fuel source of the adult brain and is transported to the CNS from the periphery via facilitative glucose transporters (McEwen & Reagan 2004). Since the brain can neither synthesize nor store glucose for extended periods of time, it is essential that proper glucose regulation is achieved in the periphery to ensure appropriate glucose transport to the CNS (Boyle et al. 1994) that may be disrupted in poorly-controlled diabetes. Dysfunctional glucose regulation and insufficient insulin availability elicits neuronal synaptic reorganization (Magarinos & McEwen 2000) and increased proliferation of astrocytes (Saravia et al. 2002). Metabolic imbalance resulting from insulin deficiency elicits measurable deficits in cognition, somatosensory and motor dysfunction (Emerick et al. 2005, Pasquier 2010). Additionally, glucose and insulin are both instrumental regulators of cognitive function (Benedict et al. 2011) further supporting the hypothesis that inefficient regulation of these two factors may contribute to cognitive deficits in diabetes phenotypes. Type 1 diabetes is more likely to be associated with psychomotor slowing and reductions in mental efficiency (Brands et al. 2005) while cognitive deficits in type 2 diabetes is often in the areas of psychomotor efficiency, attention, learning, memory and executive function (Convit 2005).
2.5.1 OUTCOMES OF DIABETIC ENCEPHALOPATHY

Various factors contributed to the development of diabetic encephalopathy resulting in structural, electrophysiological, vascular, neurochemical and neurobehavioral changes.

2.5.1.1 STRUCTURAL CHANGES

A variety of structural changes occurs in the brain of diabetic patients. Structural alterations have been found in the ventromedial hypothalamus including accumulation of glycogen, degeneration of neurons and atrophy of tanycytes (Bruehl et al. 2009). Ultrastructural abnormalities include dilated and fragmented ER, degranulated ergastoplasm, increased number of microtubuli, myelin axons, irregularities in the form of nuclei and appearance of chromatin have been observed in diabetic neurons that correlated with CNS impairment (Klein & Waxman 2003).

2.5.1.2 NEUROCHEMICAL CHANGES

The concentration of neurotransmitters has been found to be altered in diabetic brains. Animal studies have suggested a decrease in norepinephrine and serotonin content in neocortex and caudal segment of the brain stem in diabetes (Chu et al. 1986). Whereas other studies have shown an increase in the norepinephrine concentrations of the paraventricular nucleus, lateral hypothalamus, ventromedial hypothalamus and suprachiasmatic nucleus indicating that the monoaminergic system is affected in diabetic animals (Sipols et al. 1995).

2.5.1.3 NEUROPHYSIOLOGICAL CHANGES

The electrophysiological response of CNS structures to visual, auditory or somatosensory stimuli are called visual evoked potentials, brainstem auditory evoked potentials and somatosensory evoked potentials respectively and these evoked potentials are frequently used to evaluate CNS physiology (Gispen & Biessels 2000). In patients with type 1 diabetes, the interpeak latencies I–III and III–V of the brainstem auditory evoked potentials reflecting signal conduction in the pons and midbrain were found to be increased (Khardori et al. 1986). The visually evoked P100 wave latency was also found to be delayed in type 1 diabetes depicting damage to the visual cortex (Nakamura et al. 1991). Increased latencies of somatosensory evoked potentials have also been reported, although significant conduction delays were mostly limited its peripheral components. The evoked potentials are also used to explore cognitive brain functions in humans. The latencies of event related potentials (P300) reflecting the activity of cognitive and mnemonic functions in humans have been found to be increased in patients with type 1 diabetes mellitus (Tallroth et al. 1990). The relation between
CNS function and the duration of diabetes is not yet fully understood but abnormalities in evoked potentials in the type 1 diabetics suggests CNS complications or cognitive dysfunction. The exact time after disease onset and role of interventions on these abnormalities are yet to be elucidated.

2.5.1.4 NEURORADIOLOGICAL CHANGES IN THE BRAIN

Magnetic resonance imaging (MRI) studies have determined both central and peripheral atrophy in type 1 diabetic patients (Gispen & Biessels 2000). It has been suggested that the brain in patients with diabetes resembles that of normal ageing, but appears to develop at a younger age than in nondiabetic participants. Cortical atrophy has been linked to multiple severe hypoglycaemic episodes in type 1 diabetes (Fujioka et al. 1997). Focal lesions mostly involving the subcortical white matter like high intensity periventricular white matter lesions were present in one third of the scans in type 1 diabetic patients suggesting damage to the subcortical region (Matsubayashi et al. 1992). Although, these findings suggest the role of diabetes in CNS but need confirmation in future studies.

2.5.1.5 NEUROBEHAVIOURAL CHANGES

Type 1 diabetes patients have been reported to show performance deficits in various neuropsychological tests compared to controls (Gispen & Biessels 2000). Different studies have deleterious effects on general cognitive ability (i.e., intellectual functioning), psychomotor speed, attention, delayed memory and mental flexibility in diabetic subjects (Perlmuter et al. 1984). Even mild cognitive dysfunction leads to severe impairments in type 1 diabetic patients that have been seen in more demanding situations such as academic performance or vocational success (Brands et al. 2005). Several studies have reported that recurrent episodes of severe hypoglycaemia in type 1 diabetics could lead to mild cognitive deficits (Bjorgaas et al. 1997). Other complications of diabetes like retinopathy, neuropathy and nephropathy have been shown to play an important role in diabetes induced cognitive deficits as brain is susceptible to the same processes that underlie these complications (Kodl & Seaquist 2008). Studies have shown that diabetic children below age 5 are more susceptible to cognitive impairment, particularly visuo-spatial abilities, motor function, attention and memory (Kaufman et al. 1999).

2.5.1.6 SYNAPTIC PLASTICITY

Dynamic changes in synaptic strength provide a cellular basis for information storage in the brain (Gispen & Biessels 2000). A complex pattern of changes in synaptic plasticity
has been observed in hippocampus from diabetic rats that may due be to diminished expression of NMDA-dependent long term potentiation (LTP) in the CA1 and CA3 regions (Biessels et al. 1996). The severity of the LTP deficit is related to the severity of hyperglycaemia and is accentuated by ageing (Kamal et al. 2000). This plasticity deficit may be postsynaptic in nature, involving membrane excitability or the intracellular signalling cascade involved in LTP induction. Postsynaptic membrane excitability could be influenced by changes in GABA-mediated inhibition but it was found to be unaffected (Kamal et al. 1999). Diabetes could directly affect excitability by a substantial increase in adenosine sensitivity owing to the loss of nucleoside-uptake processes in the postsynaptic neuron (Biessels et al. 2002b). Also, it has been found that the affinity of glutamate for AMPA was reported to be decreased in diabetes owing to changes in the glutamate receptor GluR1 subunit37 (Gispen & Biessels 2000). Therefore, it was suggested that loss of LTP maintenance in diabetic rats was a result of disruption of Ca\(^2+\)-dependent processes that modulate postsynaptic AMPA receptors during synaptic potentiation (Artola et al. 2005). Also, it was suggested that NMDA receptor-related changes underlie the LTP deficits. As the potential reversibility of cerebral deficits in diabetes is clinically important, this issue requires further investigation.

### 2.5.1.7 DEMENTIA

There is an increasing body of evidence to support a relation between especially Type 2 diabetes and dementia (Ott et al. 1999). The risk for developing dementia doubled in diabetic patients as it may contribute to the clinical syndrome of dementia. There are many pathophysiological mechanisms through which diabetes might affect the initiation and promotion of dementia (Biessels et al. 2006). Various diabetes related pathologies like AGEs and oxidative stress can lead to dementia (Gorelick 2004). Diabetes induced vascular damage also results in dementia still the role of diabetes inducing dementia is a matter of conflict.

### 2.5.1.8 PSYCHIATRIC COMORBIDITY

The prevalence of psychiatric disorders like depression and anxiety have been found to be increased in type 1 diabetes patients, having adverse effects on cognitive functioning (Anderson et al. 2001). Increased prevalence of depression might result from an inability to cope with the stress associated with diabetes. Disturbances in glucocorticoid metabolism play a role in cognitive dysfunction in type 1 diabetes (Revsin et al. 2009) and a dysregulation of the hypothalamic–pituitary–adrenal axis activity (Bruehl et al. 2007). The relation between
cognition and depression in type 1 diabetes is complex. The co-occurrence of depression and cognitive performance could be dependent or independent features in the same underlying encephalopathy where hypothalamic–pituitary–adrenal axis plays a modulatory role.

2.5.1.9 ALZHEIMER’S DISEASE

The ε4 allele of the apolipoprotein-E (APOE) gene has been found to be associated with cognitive decline and Alzheimer's disease (Peila et al. 2002). The apolipoprotein-E genotype may also impair aspects of cognitive ability in type 1 diabetes. Especially type 1 diabetic woman with APOE ε4 performed less well on tests of non-verbal intellectual ability and executive functioning, such as planning and concept shifting compared with type 1 diabetic women without the APOE ε4 allele (Akomolafe et al. 2006).

2.5.2 PATHOGENESIS OF DIABETIC ENCEPHALOPATHY

The clinical and experimental studies have clearly demonstrated that diabetes is associated with cognitive deficits and related impairments in the cellular and molecular mechanisms of synaptic plasticity (Mijnhout et al. 2006). The pathogenesis of diabetic encephalopathy is relatively less studied and involves complex interactions of various biochemical pathways (Gispen & Biessels 2000). Multiple pathogenic factors appear to be involved causing CNS changes. The relative contribution of different factor varies between individuals, depending on characteristics such as age, type of diabetes and co-morbidity (Biessels et al. 1994).

2.5.2.1 DIRECT EFFECTS OF HYPERGLYCEMIA

Type 1 diabetes is mainly characterised by hyperglycemia and hyperinsulinemia. Hyperglycaemia leads to an increased level of glucose in the brain as in peripheral tissues.

(A) Polyol pathway

Excess glucose in brain is shunted through “polyol pathway”, by which it is converted to sorbitol by NADPH dependent aldose reductase (Fig. 4). Sorbitol is then oxidized to fructose by sorbitol dehydrogenase (Gabbay et al. 1966). Concentrations of sorbitol and fructose have been found to be increased in brain of diabetic animals (Kwee et al. 1996). Increased sorbitol levels lowered the levels of myo-inositol, leading to disturbances in phosphoinositide metabolism and impair the generation of diacylglycerol (Thomas et al. 1994). These alterations then affect the activity of protein kinases A and C in the diabetic brain thereby affecting synaptic plasticity (Klein & Waxman 2003). Polyol pathway
activation also promotes oxidative stress and nitrosative stress via depletion of NADPH resulting in decrease of GSH and GSSG (Lee & Chung 1999). It also leads to downstream events like MAPK activation, PARP activation, COX-2 activation and NFκB activation.

**Figure 4:** Aldose reductase and the polyol pathway. Aldose reductase reduces aldehydes generated by reactive oxygen species (ROS) to inactive alcohols and glucose to sorbitol, using NADPH as a co-factor. In cells where aldose reductase activity is sufficient to deplete reduced glutathione (GSH), oxidative stress is augmented. Sorbitol dehydrogenase oxidizes sorbitol to fructose using NAD+ as a co-factor.

**(B) AGE/RAGE Pathway**

Another potential toxic effect of elevated glucose levels is enhanced formation of AGEs (Fig. 5) (Giardino et al. 1994). Glycation is the nonenzymatic reaction of glucose and other saccharide derivatives with protein, nucleotides and lipids with formation of early reversible Schiff bases and later AGEs (Singh et al. 2001). Some of the AGEs formed are pentosidine and Nε-[carboxymethyl]-lysine etc. Increased amounts of AGEs have been found in the brain and spinal cord of diabetic rats. AGEs signal through the cell surface receptor called “RAGE,” thereby increasing the expression of extracellular matrix proteins, monocyte migration, vascular adhesion molecules, cytokines and growth factors (Bierhaus et al. 1998). RAGE–ligand interaction also leads to the production of intracellular ROS via the activation of an NADPH oxidase system (Wautier et al. 2001). The ROS produced in turn activate the Ras–MAPK pathway, leading to activation of NF-κB (Yan et al. 1994). Activation of NF-κB results in the transcriptional activation of many gene products thereby damaging brain.
Figure 5: Mechanisms by which intracellular production of advanced glycation end-product (AGE) precursors damages vascular cells. Covalent modification of intracellular proteins by dicarbonyl AGE precursors alters several cellular functions. Modification of extracellular matrix proteins causes abnormal interactions with other matrix proteins and with integrins. Modification of plasma proteins by AGE precursors creates ligands that bind to AGE receptors, inducing changes in gene expression in endothelial cells, mesangial cells and macrophages.

**(C) Protein Kinase C Pathway**

Hyperglycemia also leads to activation of the diacyl glycerol-protein kinase C (DAG-PKC) pathway (Fig. 6) (Xia et al. 1994). The PKC family consists of many isoforms, most of which are activated by the lipid second messenger, DAG. Under hyperglycemic conditions, there is an increase in the formation of DAG due to imbalance of phosphoinositide and pentose phosphate pathways.

Hyperglycemia also activates PKC indirectly through ligation of AGE receptors and by the influx of the polyol pathway (Brownlee 2005). Activation of various PKC isoforms results in a range of alterations in cell signaling. It has been reported that under hyperglycemic conditions, PKC-α and PKC-δ is activated by NADPH oxidase responsible for inducing Toll-like receptor TLR-2 and TLR-4 expression (Koya & King 1998). PKC activation has been also been shown to suppress nitric oxide production, which is a potent
vasodilator, by inhibiting eNOS (Geraldes & King 2010). In contrast, it increases vasoconstriction by activating endothelin-1 (ET-1), resulting in abnormal blood flow. Activation of PKC can also induce expression of the permeability-enhancing factor VEGF, contributing to blood flow and vessel permeability changes (Endemann & Schiffrin 2004). PKC also contributes to matrix protein accumulation by inducing the expression of TGF-β1, fibronectin and type IV collagen. This activation is thought to be a result of PKC-induced NO inhibition. Therefore, PKC activation leads to activation of cascade of molecules thereby damaging CNS causing cognitive deficits (Nelson et al. 2008).

![Diagram](image)

**Figure 6: Consequences of hyperglycaemia-induced activation of protein kinase C (PKC)**. Hyperglycaemia increases diacylglycerol (DAG) content, which activates PKC (β- and δ). Activation of PKC affects the expression of endothelial nitric oxide synthetase (enos), endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-B) and plasminogen activator inhibitor-1 (PAI-1) and by activating NF-KB and NAD(P)H oxidases.

**(D) Hexosamine Pathway**

The hexosamine biosynthesis pathway is a pathway of glucose metabolism that may mediate some of the toxic effects of glucose as mentioned in Fig. 7 (Brownlee 2001). Under normal metabolic conditions, 2–5% of glucose is directed into the hexosamine pathway, with the conversion of fructose-6-phosphate to glucosamine-6-phosphate. In hyperglycaemic condition activity of glyceraldehyde-3-phosphate dehydrogenase is inhibited due to overproduction of superoxide radicals, thereby diverting excess glucose into the hexosamine
pathway. The end product of this pathway is mainly UDP-N-acetylglucosamine that leads to glycosylation of many intracellular factors including transcription factors or genes like plasminogen activator inhibitor-1 and inculcate the development of the CNS complications of diabetes (Du et al. 2000).

**Figure 7: The hexosamine pathway.** The glycolytic intermediate fructose-6-phosphate (Fruc-6-P) is converted to glucosamine-6-phosphate by the enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT). Intracellular glycosylation by the addition of N-acetylglucosamine (GlcNAc) to serine and threonine is catalysed by the enzyme O-GlcNAc transferase (OGT). Increased donation of GlcNAc moieties to serine and threonine residues of transcription factors such as Sp1, often at phosphorylation sites, increases the production of factors as PAI-1 and TGF-β1. AZA, azaserine; AS-GFAT, antisense to GFAT.

**2.5.2.2 OXIDATIVE STRESS**

Toxic effects of glucose are mediated through an imbalance in the generation and scavenging of ROS (Apel & Hirt 2004). Increased concentrations of the by-products of lipid peroxidation are indicative of oxidative damage that have been demonstrated in the cerebral microvasculature and brain tissue of diabetic rats (Ulusu et al. 2003). Furthermore, the activities of superoxide dismutase and catalase, enzymes involved in the antioxidan defense of the brain were found to be decreased.
Oxidative stress cause damage to cellular proteins, membrane lipids and nucleic acids and eventually cell death (Halliwell 1991). Various mechanisms have been suggested to corroborate the formation of these free radicals (Fig. 8) including glucose oxidation that is believed to be the main source of free radicals (Giugliano et al. 1996). Glucose is oxidized in a transition-metal dependent reaction to an enediol anion that is converted into reactive ketoaldehydes and to superoxide anions (West 2000). The superoxide anion radicals undergo dismutation to hydrogen peroxide, which in the presence of transition metals can lead to production of extremely reactive hydroxyl radicals. Superoxide anions can also react with NO to form reactive peroxynitrite radicals leading to nitrosative stress. Hyperglycemia is also found to promote lipid peroxidation of LDL by superoxide-dependent pathway resulting in the generation of more free radicals (Kawamura et al. 1994).

Another important source of free radicals in diabetes is the interaction of glucose with proteins leading to the formation of AGEs. These AGEs via RAGEs, inactivate enzymes and alter their structures and functions thereby promoting free radical formation and also block the antiproliferative effects of NO (Wautier et al. 2001). AGEs also activate the transcription factor NF-κB by increasing intracellular oxidative stress, thus promoting up-regulation of various NF-κB controlled target genes (Yan et al. 1994). NF-κB also enhances production of NO, thereby causing neuronal damage via nitrosative stress (Kuhad & Chopra 2009). Various evidences also suggest that the activation of the sorbitol pathway by glucose also play an important role in generating oxidative stress in CNS of diabetic patients (Chung et al. 2003). Increased sorbitol dehydrogenase activity has been associated with altered NADPH levels thereby leading to oxidative stress via decreased GSH levels due to depletion of NADPH levels.

The four above mentioned pathogenic mechanisms cause overproduction of superoxide by the mitochondrial electron-transport chain (Rolo & Palmeira 2006). Studies have revealed that hyperglycaemia increases oxidative stress by elevating the electrochemical potential difference generated by the proton gradient across the inner mitochondrial membrane thereby increasing the lifetime of superoxide-generating electron-transport intermediates (Adam-Vizi 2005). Hyperglycaemia increases the proton gradient above the threshold value as a result of overproduction of electron donors by the TCA cycle causing a marked increase in the production of superoxide radicals in the neurons (Nishikawa et al. 2000).
Figure 8: Potential mechanism by which hyperglycaemia-induced mitochondrial superoxide overproduction activates four pathways of hyperglycaemic damage. Excess superoxide partially inhibits the glycolytic enzyme GAPDH, thereby diverting upstream metabolites from glycolysis into pathways of glucose overutilization. This results in increased flux of dihydroxyacetone phosphate (DHAP) to DAG, an activator of PKC and of triose phosphates to methylglyoxal, the main intracellular AGE precursor. Increased flux of fructose-6-phosphate to UDP-N-acetylglucosamine increases modification of proteins by O-linked N-acetylglucosamine (GlcNAc) and increased glucose flux through the polyol pathway consumes NADPH and depletes GSH.

2.5.2.3 MITOCHONDRIAL DYSFUNCTION

Brain energy requirement is largely driven to maintain ion gradients across the plasma membrane for the generation of action potentials and this is maintained by complex metabolic pathways and even slight modifications in them could result in neuronal death (Attwell & Laughlin 2001). Mitochondria are subcellular organelles that are essential for generating the energy that fuels normal cellular function as well as monitor cellular health by initiating apoptosis (Zick et al. 2009). These organelles are essential for neuronal function and suffice its energy needs by aerobic oxidative phosphorylation (Hatefi 1985). However, oxidative phosphorylation is process that also generates endogenous toxic free radicals, including hydrogen peroxide, hydroxyl and superoxide ions (Chen et al. 2003) (Fig. 9).

Under normal condition, electrons accumulate in complex I and coenzyme Q, where they are donated to molecular oxygen to give superoxide anions that can be detoxified by the mitochondrial manganese superoxide dismutase (MnSOD) to give H$_2$O$_2$ that, in turn, can be converted to H$_2$O by glutathione peroxidase (GPx) (Kushnareva et al. 2002). However,
superoxide anions in the presence of nitric oxide (NO) get converted to peroxynitrite by nitric oxide synthase (NOS) (Mastrocola et al. 2005). H₂O₂ in the presence of reduced transition metals can also be converted to toxic hydroxyl radicals via Fenton and/or Haber Weiss reactions (Thomas et al. 2009). Intriguingly, if the amount of free radical species produced overwhelms the neuronal capacity to neutralize them and oxidative stress occurs, followed by mitochondrial dysfunction and neuronal damage thereby leading to cognitive dysfunction (Lin & Beal 2006). ROS generated by mitochondria have several cellular targets including mitochondrial components themselves (lipids, proteins and DNA). The lack of histones in mitochondrial DNA (mtDNA) and diminished capacity for DNA repair render mitochondria an easy target to oxidative stress events (Kroemer et al. 1998). Mitochondrial dysfunction and the resulting energy deficit trigger the onset of neuronal degeneration and death.

**Figure 9: Production of superoxide by the mitochondrial electron-transport chain.**

Increased hyperglycaemia-derived electron donors from the TCA cycle (NADH and FADH₂) generate a high mitochondrial membrane potential (ΔµmH⁺), by pumping protons across the mitochondrial inner membrane. This inhibits electron transport at complex III, increasing the half-life of free-radical intermediates of coenzyme Q (ubiquinone), which reduce O₂ to superoxide.

Increased oxidative stress has been implicated in the diabetic brain (Mastrocola et al. 2005) as several studies have suggested that it leads to oxidative injury of dorsal root ganglion neurons, mitochondria being a specific target (Schmeichel et al. 2003). Loss in the control of glucose homeostasis also leads to the decrement in oxidative phosphorylation efficiency (Taylor et al. 2005). It has been shown that diabetes leads to lactic acidosis, poor
respiration and marked defects in mitochondrial morphology and respiratory chain complex I and IV activities (Kamboj & Sandhir 2010).

Diabetes mellitus leads to functional and structural changes in the brain, which appear to be most pronounced in the elderly (Biessels et al. 2002b). It has been shown that mitochondrial function declines with aging and diabetes (Moreira et al. 2007). This is due to impairment of the respiratory chain and an uncoupling of oxidative phosphorylation in brain mitochondria in diabetic model (Mastrocola et al. 2005). The maintenance of oxidative phosphorylation is extremely important in the brain since about 90 % of the ATP required for the normal functioning of neurons is provided by mitochondria (Calabrese et al. 2001). Mitochondrial impairment results in neurodegeneration and loss in neuronal metabolic control as CNS requires a large amount of ATP for the transmission of impulses along the neural pathway.

Recent findings indicated that insulin is a major regulating factor of mitochondrial oxidative phosphorylation (Szendroedi et al. 2011). Insulin selectively stimulates mitochondrial protein synthesis and activates mitochondrial enzyme activity and its deprival decreases mitochondrial oxidative phosphorylation efficiency in diabetes. Also, IGF-1 has been shown to protect from hyperglycemia-induced oxidative stress and neuronal injuries by regulating ΔΨm, possibly by the involvement of uncoupling protein 3 (UCP3) (Gustafsson et al. 2004). Diabetes has also been shown to accentuate depolarization of the mitochondrial inner membrane in sensory neurons (Huang et al. 2003). Type 1 diabetes also leads to reduced mitochondrial antioxidant defenses (CoQ9 content) (Moreira et al. 2006). Insulin and IGFs plays a pivotal role in protein kinase B-mediated expression of Bcl2 protein that prevents the escape of ROS by opposing the oxidative-stress-induced pro-apoptotic action of Bax (Rajah et al. 2002). Also, insulin has been shown to protect cortical neurons against oxidative stress by modulating redox cycle (Filomeni et al. 2002). The role of mitochondria in diabetes induced CNS complications is complex and needs to be further studied.

2.5.2.4 CALCIUM HOMEOSTASIS

Disturbances in the calcium homeostasis may present a pathway in the multifactorial pathogenesis of neurological complications of diabetes, which involves vascular changes, oxidative stress and non-enzymatic protein glycation (Levy 1999). A prolonged, small increase in basal cytosolic Ca$^{2+}$ levels has been seen in sensory neurons of diabetic animals.
Learning deficits in diabetes develop in association with distinct changes in synaptic plasticity in hippocampus, depending on diabetes duration and severity (Kamal et al. 2000). Alterations in neuronal Ca\(^{2+}\) homeostasis play an important role in cognition and synaptic plasticity that have been observed in experimental diabetes, together with alterations in other signal transduction cascade components such as protein kinase A, protein kinase C, cAMP, phospholipase C, phospholipase A2, diacylglycerol and inositol phosphate (Biessels et al. 2002a). Alterations in glutamatergic neurotransmission also appear to be involved with disturbances of calcium homeostasis (Moulder et al. 2003). After prolonged diabetes, the level as well as phosphorylation of the NR2B subunit of the NMDA receptor is decreased by Ca\(^{2+}\)/calmodulin-dependent protein kinase II (Di Luca et al. 1999). This reduces the NMDA receptor mediated currents in hippocampal pyramidal neurons thereby affecting synaptic plasticity.

Mitochondria also serve as high capacity Ca\(^{2+}\) sinks, which aid in maintaining cellular Ca\(^{2+}\) homeostasis that is required for normal neuronal function (Brini et al. 2013). Also, excessive Ca\(^{2+}\) uptake into mitochondria results in ROS overproduction, ATP synthesis inhibition, cytochrome c release and induction in mitochondrial permeability transition (MPT) (Celsi et al. 2009). MPT regulates the mitochondrial membrane potential (ΔΨm), coupling of the electron transport system, mitochondrial swelling and release of proapoptotic proteins (Zoratti & Szabo 1995). Ca\(^{2+}\), inorganic phosphate, oxidative stress and low inner membrane potential promote the onset of MPT, whereas cyclosporin A (CsA), Mg\(^{2+}\), ADP and the existence of a high membrane potential oppose the onset. Mitochondria being an important cytoplasmic Ca\(^{2+}\) buffer avoid the increase of Ca\(^{2+}\) above a critical value. In oxidative stress conditions, an increase in intracellular Ca\(^{2+}\) concentration occurs and the cytosolic Ca\(^{2+}\) levels modulates several intracellular signalling pathways, including PKC-\(\alpha\) and calmodulin-dependent signalling, which have been implicated in apoptotic processes (Orrenius et al. 2003). The maintenance of Ca\(^{2+}\) homeostasis is tightly coupled to the rates of oxidative phosphorylation and the generation of ROS (Ermak & Davies 2002). It was observed that diabetes decreases the capacity of mitochondria to accumulate Ca\(^{2+}\), a favourable intracellular environment for MPT opening (Moreira et al. 2003). The MPT opening results in osmotic swelling of mitochondria leading to structural changes of these organelles. High glucose concentrations induce elevated levels of oxidative phosphorylation, resulting in ROS overproduction and calcium release that lead to changes in mitochondrial structure and function (Siesjo 1994). It was also studied that insulin is capable to increase the capacity of mitochondria to accumulate Ca\(^{2+}\) suggesting a role of insulin in Ca\(^{2+}\) homeostasis
(Huang et al. 2003). Thereby, disruption of insulin as in type 1 diabetes has serious consequences on calcium homeostasis and thereby on CNS.

![Diagram of cerebrovascular complications]

**2.5.2.5 CEREBROVASCULAR COMPLICATIONS**

Diabetes has been associated with both structural and functional alterations of the cerebral vascular system affecting cognitive functions (Brands et al. 2004). Brain autopsy of diabetic patients clearly demonstrates structural abnormalities in the microvessels that include thickening of capillary basement membranes and decreased capillary density (Mooradian 1997). Type 1 diabetes has also been associated with regional alterations in cerebral blood flow and vascular reactivity. Cerebral vasoreactivity and accompanying changes in blood flow are important compensatory mechanisms during conditions such as hypoglycaemia, hypotension, hypoxia and hypercapnia and their loss have detrimental effects on the brain (McCall 2002). The etiology of brain dysfunction in diabetes is primarily related to small vessel disease however it is also possible that abnormalities in the structure and function of BBB could contribute (Horani & Mooradian 2003). The recent findings suggested that diabetic patients appear to have a leaky BBB causing leukoaraiosis that may be associated with cognitive impairment and increases the risk of dementia (Wardlaw et al. 2003).
Review of Literature

(A) Blood Brain Barrier (BBB)

The CNS being the most critical and sensitive system in the human body, necessitates a highly regulated extracellular environment, wherein the concentrations of ions such as Na\(^+\), K\(^+\) and Ca\(^{2+}\) as well as different metabolites must be maintained within very narrow ranges for proper neuronal function (Rolfe & Brown 1997). CNS also needs to be protected against various neurotoxic chemicals that are readily metabolized and excreted without harm to peripheral organ systems (Hawkins & Davis 2005). Therefore, a dynamic regulator of ion balance, a facilitator of nutrient transport and a barrier to potentially harmful molecules is essential that acts as an interface between the CNS and the peripheral circulatory system. This homeostatic and dynamic function of the cerebral microcirculation is performed by BBB that performs all of these functions.

Figure 11: Structure of blood brain barrier (BBB). The BBB is formed by cerebral endothelial cells ensheathing the vessels of the brain surrounded by pericytes, astrocytes and neurons.

BBB is a selective diffusion barrier that consists of cerebral microvascular endothelium characterized by the presence of tight junctions and lack of fenestrations. Fig. 11 shows a schematic cross-sectional representation of a typical cerebral capillary. The circumference of the capillary lumen is enclosed by a single endothelial cell that are characterized by increased mitochondrial content (Oldendorf et al. 1977), minimal pinocytotic activity (Sedlakova et al. 1999) and the presence of tight junctions (Kniesel &
Endothelium is mainly surrounded by pericytes that together are ensheathed by the basal lamina, a membrane 30 to 40 nm thick composed of collagen type IV, heparin sulfate proteoglycans, laminin, fibronectin and other extracellular matrix proteins (Farkas & Luiten 2001). The basal lamina is further encircled by astrocyte end-feet which ensheath cerebral capillaries.

**(B) Neurovascular unit**

It has been proposed that the microvascular endothelium, astrocytes, pericytes, neurons and extracellular matrix constitute a “neurovascular unit” constituting BBB (Wang et al. 2004, Neuwelt 2004). The neurovascular unit helps in understanding brain responses to cerebrovascular pathology as well as the multiple pathways by which cerebral microvascular permeability could be regulated by drugs or disease (Lo et al. 2004).

**(i) Astrocytes**

Astrocytes are critical in the development and maintenance of BBB characteristics and morphology (Davson & Oldendorf 1967). Astrocytes may act as intermediaries in conjunction with neurons in the regulation of cerebral microvascular permeability via dynamic Ca\(^{2+}\) signaling between astrocytes and the endothelium via gap junctions and purinergic transmission (Ballabh et al. 2004). Astrocytic foot processes also contain water channels (Aquaporin-4) that allow water uptake and contribute to brain swelling (Braet et al. 2001, Zonta et al. 2003). Astrocytic end-feet tightly ensheathe the pericytes and endothelium vessel wall and release trophic factors that are critical for the induction and maintenance of the BBB.

**(ii) Pericytes**

Involvement of pericytes at the BBB has yet to be further studied but the presence of contractile proteins in them has indicated that they may play a role in regulation of capillary blood flow (Bandopadhyay et al. 2001). Pericytes have also been found to stabilize the formation BBB (Ramsauer et al. 2002). They have also been shown to migrate away from brain microvessels in rapid response to stress situation associated with increased BBB permeability (Gonul et al. 2002, Dore-Duffy et al. 2000). Pericyte-derived angiopoetin can induce endothelial expression of occludin, a major constituent of BBB tight junction protein, indicating that they are involved in the induction and maintenance of barrier properties in the cerebral endothelium (Hori et al. 2004).
(iv) Neurons

The dynamic nature of neurons and its aggressive metabolic needs are served by BBB. The communication between neurons and the vasculature regulate blood flow as well as BBB permeability. Cerebral microvascular endothelium in association with astrocytic processes are directly innervated by noradrenergic, serotonergic (Cohen et al. 1997), cholinergic (Tong & Hamel 1999) and GABA-ergic neurons (Vaucher et al. 2000) as well as others (Kobayashi et al. 1985). Neurons are critical in regulation of critical aspects of BBB function but neuronal role in the development of the BBB phenotype has not yet been demonstrated.

(v) The extracellular matrix

The extracellular matrix of the basal lamina also interacts with the cerebral microvascular endothelium. The extracellular matrix serves as an anchor for the endothelium via interaction of laminin and other matrix proteins with endothelial integrin receptors (Hynes 1992). Such cell-matrix interactions can stimulate a number of intracellular signaling pathways (Tilling et al. 2002). Matrix proteins like MMPs can influence the expression of endothelial tight junction proteins playing a major role in BBB maintenance (Savettieri et al. 2000).

(C) Junctions of the BBB

BBB is characterized by the presence of a junctional complex in the interendothelial space that includes adherens junctions (Schulze & Firth 1993), tight junctions (Vorbrodt & Dobrogowska 2003) and gap junctions (Simard et al. 2003). Adheren junctions and tight junctions play an important role in restricting permeability across the endothelium while gap junctions mediate intercellular communication (Bazzoni & Dejana 2004).

(i) Adheren junction

Adheren junctions are ubiquitous in the vasculature and mediate the adhesion of endothelial cells to each other playing important role in vascular growth as well as remodeling, cell polarity and the regulation of paracellular permeability (Brown & Davis 2005). The primary component of adheren junctions is vascular endothelial-cadherin, a Ca²⁺ regulated protein that mediates cell-cell adhesion (Vincent et al. 2004). The cadherin further binds to β-catenin and plakoglobin which in turn binds to the actin cytoskeleton thereby stabilizing the adheren junction complex (Watabe-Uchida et al. 1998).
(ii) Tight junctions

Tight junctions are elaborate structures that span the apical region of the intercellular cleft of epithelial and endothelial barrier tissues (Fig. 12). Tight junctions confer low paracellular permeability and high electrical resistance (Romero et al. 2003). They function both as a “zipper” that affects separation of the apical and basolateral cell membranes, enabling asymmetric distribution of membrane constituents and a “fence” that limits paracellular permeability. Tight junctions are composed of transmembrane proteins that form the primary seal linked via accessory proteins to the actin cytoskeleton (Vorbrodt & Dobrogowska 2003). The transmembrane components of the tight junction at the BBB include junctional adhesion molecule (JAM)-1 (Del Maschio et al. 1999), occludin and the claudins.

1. JAM-1- It is a 40-kDa member of the IgG superfamily and mediate the early attachment of adjacent cell membranes via homophilic interactions (Dejana et al. 2000). JAM-1 is composed of a single membrane-spanning chain with a large extracellular domain (Martin-Padura et al. 1998). JAM-1 role in the mature BBB is still largely unknown but it may regulate the transendothelial migration of leukocytes (Del Maschio et al. 1999).

2. Occludin- It is a 60 to 65 kDa protein, has four transmembrane domains with the carboxyl and amino terminals oriented to the cytoplasm and two extracellular loops that span the intercellular cleft (Furuse et al. 1998). It is present in a continuous pattern along the cell margins in the cerebral endothelium (Hawkins et al. 2004). Occludin increases electrical resistance in tight junction containing tissues (McCarthy et al. 1996). Occludin has multiple sites for phosphorylation on serine and threonine residues which is involved in the regulation of its association with the cell membrane (Kale et al. 2003). The cytoplasmic C-terminal domain is likely involved in the association of occludin with the cytoskeleton via accessory proteins; ZO-1 and ZO-2 (Fanning et al. 1998). Decreased expression of occludin is associated with disrupted BBB function in a number of disease states (Huber et al. 2001).

3. Claudins- Claudins are 20-24 kDa proteins having membrane topography as that of occludin but no sequence homology (Huber et al. 2001). Claudins interact via homophilic and heterophilic interactions between cells (Furuse et al. 1999). Claudins form the primary seal of the tight junctions and occludin acts as an additional support structure. Cerebral microvascular endothelium consists of claudin-1, -3 and -5.
4. Membrane-Associated Guanylate Kinase-Like Proteins- In addition to the transmembrane components of the tight junctions, there are several accessory proteins that associate with them in the cytoplasm. These include members of the membrane-associated guanylate kinase-like (MAGUK) homolog family. MAGUK proteins have been involved in the coordination and clustering of protein complexes to the cell membrane and in the establishment of specialized domains within the membrane (Gonzalez-Mariscal et al. 2000). Three MAGUK proteins have been identified at the tight junctions: ZO-1, ZO-2 and ZO-3. ZO-1 is a 220-kDa phosphoprotein expressed in endothelial cells and has been found to be associated with the tight junctions (Stevenson et al. 1986), adheren junction (Itoh et al. 1993) and gap junction proteins (Toyofuku et al. 1998). ZO-1 links transmembrane proteins of the tight junctions to the actin cytoskeleton (Fanning et al. 1998). This interaction is likely critical to the stability and function of the tight junctions because dissociation of ZO-1 from the junctional complex is often associated with increased permeability (Mark & Davies 2002). ZO-1 may also act as a signaling molecule that communicates the state of the tight junctions to the interior of the cell or vice versa. ZO-1 has been shown to localize to the nucleus under conditions of proliferation and injury (Gottardi et al. 1996). ZO-2, a 160-kDa phosphoprotein has a high-sequence homology with ZO-1 (Gumbiner et al. 1991). ZO-2 like ZO-1 binds to both structural constituents of the tight junctions and signaling molecules such as transcription factors and localizes into the nucleus during stress and proliferation (Benzos et al. 2004). ZO-3 is a 130-kDa homolog that has been found in some tight junction containing tissues but not in BBB (Inoko et al. 2003).

5. Other Accessory Proteins- Other accessory proteins of the BBB include cingulin, AF-6 and 7H6. Cingulin is a 140- to 160-kDa protein that associates with ZOs, JAM-1 and myosin, hypothesized to mediate interactions between the cytoskeleton and the tight junctions via force transduction (Citi et al. 2012). AF-6, a 180-kDa protein with two Ras-associating domains, interacts with ZO-1. This interaction is inhibited by Ras activation, indicating that disruption of the ZO-1/AF-6 complex may be critical in the modulation of tight junctions via pathways that involve Ras (Yamamoto et al. 1999). The function of 7H6 is not known, but this 155-kDa protein reversibly dissociates from the tight junctions complex under conditions of ATP depletion.
Figure 12: Molecular composition of endothelial tight junctions. Occludin and Claudins are important membranous components. The junctional adhesion molecules (JAMs) and the endothelial selective adhesion molecule (ESAM) are members of the immunoglobulin superfamily. Within the cytoplasm are many first-order adaptor proteins included zonula occludens 1, 2 and 3 (ZO-1–3) and Ca2+-dependent serine protein kinase (CASK). Among the second-order adaptor molecules, cingulin is important. The most important molecule of endothelial adherens junctions is vascular endothelial cadherin (VE-cadherin).

Compounds cross the BBB in several ways (Lossinsky & Shivers 2004). These are by: 1) passive diffusion, particularly for lipid-soluble substances; 2) facilitative and energy-dependent receptor mediated transporters, e.g., transferrin receptor, LDL-receptor; 3) Carrier mediated transporters which provide essential brain nutrients, e.g., GLUT-1 glucose, CAT-1 basic amino acids, LAT-1 neutral amino acids, EAAT-1 acidic amino acids; and 4) absorptive transeptosis, e.g., albumin. Additionally there are important proteins like P-glycoprotein (MDR-1), BCRP, OAT and OCT families that efflux compounds from the endothelial membrane and basolateral membrane.

2.5.2.6 ALTERATIONS IN BBB IN DIABETES

Failure in BBB lead to progression of several neurological diseases, indicating that the BBB play an important role in CNS functions (Zlokovic 2008) (Fig. 13). Whereas, role of BBB in diabetes induced CNS complications is still controversial.

(A) Changes in permeability

Diabetes is associated with considerable alterations in BBB function (Chehade et al. 2002). It was also found that BBB leakage is one of the major debilitating factors causing
cognitive decline in diabetic patients (Huber 2008). Various studies have suggested that BBB remains intact (Dai et al. 2002) and others suggest that BBB permeability is increased in diabetes (Iwata et al. 1999). It was found that albumin but not IgG or C3 enters the cerebral cortex after the onset of diabetes (Stauber et al. 1981). Diabetes also resulted in larger inulin spaces in certain areas of the cerebrum including mediobasal hypothalamus, mediodorsal hypothalamus and periaqueductal gray (Lorenzi et al. 1986). Furthermore, extravasation of Evans blue albumin is more pronounced in the brains of diabetic rats compared to controls after adrenalin-induced acute hypertension (Oztas & Kucuk 1995). Serum S100B and NSE (CNS proteins) are also reported to be significantly increased in both type 1 and type 2 diabetic subjects, implying that diabetes in humans may be associated with alterations in the integrity of the BBB (Hovsepyan et al. 2004). Ischemic injury in diabetic rats demonstrated that hyperglycemia aggravated BBB permeability, edema formation and neurological manifestations because of extravasation of inflammatory cells and fluid into the brain tissue (Kamada et al. 2007). In addition, increased BBB permeability and white matter hyperintensities have been detected in diabetic subjects by gadolinium magnetic resonance imaging, suggesting alterations in BBB integrity (Starr et al. 2003). However, BBB permeability to horseradish peroxidase, sucrose or cytochrome c is not altered in diabetic rats. STZ induced diabetes has been shown to increase BBB permeability and the changes were region specific in cortex, hippocampus, midbrain and basal ganglia (Huber et al. 2006). These alterations could be the result of changes in physical and chemical properties of the BBB (Chehade et al. 2002).

(B) **Structural and molecular changes**

BBB is made of basement membrane, astrocytic foot processes and components of the tight junctions such as occludin and ZO-1. Basement membrane of vessels in the hypothalamic arcuate nucleus and the occipital/frontal cortices were found to be thickened as observed in diabetes and this thickening was region specific (Mooradian 1997). There was also degeneration of pericytes in the hypothalamus and cortex after diabetes. Furthermore, the density of plasmalemnal vesicles almost doubled that account for the alteration in BBB permeability seen in diabetes. The astrocytic end-feet were found to be swollen and contained mitochondria having longitudinal rearrangement of their cristae in diabetes. Since astrocytic end-feet are actively involved in the maintenance of the BBB and thickening of the basement membrane such abnormalities affect both barrier and transport properties in diabetes.
It was also found that occludin mRNA and protein content were significantly reduced in diabetes suggesting that it affects the translation of the occludin mRNA. It has been found that hyperglycemia increases BBB permeability via loss of tight junction proteins and increased MMP activity. Diabetes-related changes in expression of cerebral occludin and ZO-1 has also been reported (Chehade et al. 2002). Matrix metalloproteinases (MMPs) were also found to be increased in diabetic rats, concurrently with a decreased production of the BBB tight junction proteins occludin and ZO-1 (Hawkins et al. 2007). Furthermore, decrease in the BBB function allows leakage of serum proteins that could result in brain edema formation (Dietrich et al. 1993). Thus, it is quite likely that hyperglycemia associated with diabetes mellitus may alter the brain microvasculature leading to brain edema formation through modification of the BBB function (VanGilder et al. 2009). Diabetes-related cerebral edema is likely multifactorial and not simply the result of either cerebral oligemia (low cerebral blood flow) or cerebral hyperemia (high cerebral blood flow) alone, but rather the result of an interplay of complex pathophysiological processes involving the brain.

**(C) Changes in BBB specific transport**

A number of studies have examined BBB glucose transport in hyperglycaemia but the results are controversial (Simpson et al. 1999). BBB glucose transport was downregulated in chronic hyperglycaemia (Pardridge et al. 1990). Reduced BBB glucose transport could be an adaptive change to protect the CNS from long-term glucotoxicity. These changes in BBB glucose transport were reported to be due to reduced GLUT-1 concentration following hyperglycemia (Choi et al. 1989). These results support the hypothesis that cerebral glycopenia is responsible for hypoglycemic symptoms in diabetes. Additionally, it was stated that change in glucose transporter density in the cerebral microvessels does not necessarily mean a corresponding change in glucose transport at the BBB (Kumagai 1999). The glucose transporter density is a relatively static value which changes only slowly in response to an altered homeostatic environment, whereas glucose transport is a dynamic state.

It was also found that transport of choline (the substrate for the neurotransmitter acetylcholine), was significantly reduced in diabetic animals followed by reduced synthesis of acetylcholine (Mooradian 1987). Influx of phenylalanine and several other basic amino acids (e.g. tryptophan, tyrosine and methionine) was also found to be reduced in diabetic situation (McCall et al. 1982). These observations, however, might not necessarily reflect an alteration of the transport at the BBB. Reduced influx could be responsible for lowering of some essential amino acids, with possibly deleterious consequences for brain functions.
Vascular abnormalities contribute to dysfunction in the BBB, impair delivery of essential nutrients to neuronal tissue and predispose the animal to cerebral hypoxic injury during reduced perfusion pressure or poor ventilation (Ballabh et al. 2004). Structural changes in the cerebral microvessels in diabetes include reduced density of cortical capillaries, increased capillary basement membrane thickening, arteriovenous shunting, paucity of cortical capillaries and calcium depositions in microvessel walls of diabetic animals (Mooradian 1997). It was reported that there was a significantly higher concentration of lipid peroxidation by-products, mainly conjugated dienes after diabetes that gets accumulated in cerebral microvessels (Mooradian & Smith 1992). However, no change appeared in the composition of cholesterol, phospholipids and fatty acids after diabetes. Diabetes can also affect the protein composition of cerebral microvessels, which may also contribute to diabetes related changes in the BBB (Mooradian et al. 1994). It was also found that the level of glycosylation of cerebral microvessel protein mixture was significantly increased after diabetes and many of the proteins were significantly altered thereby affecting cerebral vessel.
Diabetes induced changes on the brain mainly include lacunar infarct, ischemic stroke and vascular dementia (Karapanayiotides et al. 2004). Many of the debilitating consequences associated with diabetes result from prolonged vascular dysfunction (VanGilder et al. 2009). Evidence suggests that hyperglycemia-induced oxidative stress plays a primary role in the vascular complications most commonly associated with diabetes (Figueroa-Romero et al. 2008). Oxidative stress results from an imbalance between generation of ROS and antioxidant capacity. ROS at low concentrations play important physiological roles as signalling molecules and contribute to localized regulation of vascular tone (Faraci 2006). Excess ROS has a number of detrimental implications on vascular function, including depletion of nitric oxide bioavailability, increased nitrosative stress, vascular remodeling, depletion of antioxidant enzymes and impaired vascular coupling (Valko et al. 2007). Hyperglycemia-induced ROS may contribute to dysregulation of the BBB, which could disrupt the neuronal microenvironment and translate to cognitive deficits. The BBB is a heterogeneous structure in which the vasculature in certain regions of the brain is more vulnerable to oxidative damage and neurovascular uncoupling (Chrissobolis & Faraci 2008).

Generation of excess ROS has been shown to increase BBB permeability by alteration in tight junction protein expression and increased vascular remodelling (Haorah et al. 2007). Also, ROS certainly have a role in altering the basement membrane during diabetes (Balakumar et al. 2009). In fact, vascular remodelling is a hallmark indicator of diabetes-induced angiopathies with characteristic thickening of the basement membrane and hardening of the blood vessels (Kolluru et al. 2012). In addition, increased vascular permeability and changes in tight junction protein expression during diabetes are well documented in the retina (Antonetti et al. 1999). Elevated blood glucose levels lead to decreased nitric oxide bioavailability, which contributes to unmet metabolic demand of neurons, impaired neurovascular coupling and loss of vascular reactivity (Honing et al. 1998). Dysfunction of metabolic pathways in brain vasculature during diabetes has been hypothesized to precede noticeable cognitive deficits (Ryan et al. 2003). Impairment of BBB function during diabetes may be a predisposing factor for the increased incidences of vascular dementia and cerebrovascular disease in people with diabetes.

2.5.2.7 HYPOGLYCEMIA

Severe and prolonged episodes of hypoglycaemia in diabetes provoke brain damage through uncontrolled release of excitatory amino acids like glutamate and aspartate, which
trigger calcium influx, leading to activation of proteolytic enzymes, thereby causing neuronal damage (Perros et al. 1997). Studies have shown that the severity of the brain damage is dependent on the duration of hypoglycaemia (Ferguson et al. 2003). Hypoglycaemia and its associated regulatory hormonal responses lead to acute rise in haematocrit and blood viscosity that alter capillary blood flow thereby causing microvascular complications (Reichard et al. 1993). While some studies found no association between frequency of severe hypoglycaemia and cognitive impairment. Future studies have to be targeted to study the adverse effects of hypoglycaemia on brain.

2.5.2.8 INSULIN SIGNALLING

Insulin receptors are widely distributed in the brain. Insulin signalling through its cerebral receptors influences the regulatory processes associated with food intake, body weight and can act as a “neuromodulator,” influencing the release and reuptake of neurotransmitters (Biessels et al. 2004). The insulin receptors are concentrated in specific brain regions including the olfactory bulbs, limbic system, hypothalamus and hippocampus, whereas insulin itself is particularly abundant in the hypothalamus and olfactory bulb. Although glucose uptake by the brain is mainly insulin-insensitive, insulin does affect cerebral glucose utilisation to some extent.

Impairments in the insulin signalling pathway in the brain have been implicated in diabetes and ageing. Diabetes and its treatment with insulin are likely to affect cerebral insulin levels and insulin signalling (Liu et al. 2011). Insulin signalling is disturbed both in type 1 and type 2 diabetes. The nature of the relation between hyperinsulinaemia and cognitive function in type 1 or type 2 diabetes in humans is unclear. Differences in insulin action in the brain between patients with type 1 and type 2 diabetes explain the distinctive cognitive profiles of these two conditions. Acquisition of information over time (i.e., learning) and consolidation of information for long-term storage seem to be relatively spared in type 1 diabetes compared with type 2 diabetes. These two cognitive domains are critically dependent on the hippocampus and this structure has a relatively high density of insulin receptors and therefore is extra vulnerable for defects in insulin action.

2.5.2.9 INFLAMMATION AND DIABETES

Inflammation is a biological process that generates a repertoire of molecules in response to alterations in tissue integrity to restore tissue homeostasis through the induction of various repair mechanisms. Proper regulation of these mechanisms is essential to prevent
uncontrolled amplification of the inflammatory response and disease development. Sensing of pathogen-associated molecular patterns and damage-associated molecular pattern is ensured by a complex set-up of pattern-recognition receptors like RAGE. RAGE activation triggers many intracellular signaling pathways, including kinases (e.g., MAP kinases, PI3 kinase), adaptors, transcription factors (NFκB) and activator protein-1. Such signaling molecules further control the expression of cytokines, chemokines, enzymes, growth factors and tissue repair molecules (Wellen & Hotamisligil 2005). However, in certain situations restoration may not occur adequately, resulting in cellular stress and amplified inflammatory response leading to significant alterations of tissue functions. Diabetes and neurodegenerative diseases are the main pathological processes associated with such inflammatory changes (Lobner & Fuchtenbusch 2004).

Inflammation is coupled with significant alterations in redox equilibrium, due to the associated enhancement of oxidant generation and mitigating oxidative stress has proved to be a potentially useful anti-inflammatory strategy (Celik & Erdogan 2008). Activation of NFκB controls the expression of many genes associated with innate immune responses and also a redox sensitive nuclear factor involved in the control of a large number of cellular processes (Ramasamy et al. 2005). It has been suggested in many studies that diabetes leads to NFκB activation thereby increasing the expression of proinflammatory cytokines. Moreover, NFκB pathway triggers neuronal apoptosis thereby impairing cognitive function in experimental diabetes. Activated NFκB can also induce cytotoxic products that exacerbate inflammation and oxidative stress promoting apoptosis induced neuronal dysfunction. Type 2 diabetes has been found to activate IKKβ/NFκB thereby inducing proinflammatory pathway. In this disease, intracellular oxidative stress and mitochondrial dysfunction mediate hypothalamic NFκB activation which further activates intracellular stresses such as mitochondrial oxidative stress and ER stress (Dufey et al. 2014). Type 2 diabetes also results in ER stress thereby activating cellular inflammatory pathways which impair neuronal functions (Ozcan et al. 2006). Moreover, brain ER stress promotes NF-κB activation in the development of central metabolic dysregulation associated with inflammatory pathways. Exposure to high glucose induces ER stress by the generation of free radicals, aberrant protein glycosylation, or increased membrane and protein turnover (McAlpine et al. 2010). It has been found that diabetes leads to increased expression of C/EBP homology protein (CHOP), the prominent mediator of the ER stress-induced apoptosis causing synaptic and neuronal impairment and promote the diabetic cognitive impairment (Oyadomari et al. 2002).
2.6 **DIABETES - A PRECURSOR FOR OTHER COMPLICATIONS**

Diabetes has been found to exacerbate various other complications like stroke, ketoacidosis, Alzheimer’s disease that further deteriorate the condition. These complications increase morbidity and mortality in diabetic individuals.
2.6.1 CEREBRAL ISCHEMIA AND HYPERTENSION

Diabetes has been found to aggravate brain damage in both experimental and clinical stroke subjects (Pulsinelli et al. 1983). Diabetes accelerates maturation of neuronal damage, increases infarct volume and induces post-ischemic seizures. Focal ischemia in normoglycemic rats leads to brain damage in a delayed fashion but in pre-ischemic hyperglycaemic rats the damage is more severe. The presence of hypertension is also associated with increased morbidity and mortality in diabetic patients. Studies have established that diabetes and hypertension are independent risk factors for the increased BBB permeability (Capes et al. 2001). Acute and chronic hypertension increases the permeability of BBB. Defects in the synthesis or release of NO might play an important role in the endothelial dysfunction of diabetic hypertensive vessels (Li et al. 2010). NO has an important role in the regulation of vascular tone and the impairment of NO is associated with increased vascular reactivity to constrictors, enhanced platelet/leukocyte adhesion and atherogenesis. Type 1 diabetes could affect NO production and function through different pathways including iNOS expression and these alterations could be implicated in the development of vascular complications associated with type 1 diabetes. The mechanisms underlying diabetes-related changes of ischemic brain damage and hypertension are unclear. Cerebrovascular changes, oxidative stress, inflammation, apoptosis are the key pathways that are believed to play an important role in the pathogenesis of diabetes leading to cerebral ischemia and hypertension (Muranyi et al. 2003). Type 1 diabetic animals show definite alterations in structure, neurotransmitters, electrophysiology, cognitive function, neuronal density and apoptotic activity. These results seem to suggest that “primary diabetic encephalopathy” is an identifiable complication in type 1 diabetes and that exaggerated brain damage occurs consequent to ischemia and hypertension in type 1 diabetes.

2.6.2 ALZHEIMER’S DISEASE

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a selective neuronal cell death associated with two hallmark pathological lesions: the intracellular neurofibrillary tangles (NFTs) and extracellular amyloid deposits in the form of senile plaques (Craig-Schapiro et al. 2009). The etiological events leading to AD pathogenesis are unclear. It has been thought that AD occurs due to the accumulation of aggregated neurotoxic Aβ in specific brain regions (hippocampus and cerebral cortex), triggering an inflammatory response, neuronal cell death and gradual cognitive decline. The islet of Langerhans in type 2 diabetes is characterized by β-cell loss and islet amyloid derived from islet amyloid
polypeptide (IAPP). Furthermore, it has been reported that degeneration of pancreatic islets is also associated with NFTs formation (Kroner 2009). Similarly to AD, the incidence of type 2 diabetes strongly increases with age. Altogether these findings implicate a close biological relationship between type 2 diabetes and AD. It has been found that insulin affects several brain functions and regulates the metabolism of Aβ and tau proteins (Steen et al. 2005). Furthermore, it has been suggested that desensitization of the neuronal insulin receptors and signalling events in AD, leads to a reduction in acetylcholine and a corresponding decrease in cerebral blood flow. These abnormalities result in chronic and increasing deficits in brain oxidative metabolism. Due to the increasing number of studies demonstrating a connection between diabetes and AD, efforts have been developed to elucidate the exact mechanism(s) underlying this connection.

2.6.3 DIABETIC KETOACIDOSIS

Ketosis is a state characterized by elevated serum levels of ketone bodies (Lebovitz 1995). In addition to hyperglycemia, type 1 diabetics frequently experience ketosis due to insulin deficiency. This condition is more common and severe in patients with type 1 versus type 2 diabetes, but it may exacerbate insulin resistance in type 2 diabetes. Ketones can cross BBB and may be used as an alternative to glucose as an energy source in the brain. Ketone accumulation in the brain may be important because ketones have been found to have direct effects on the brain microvascular endothelium and they may alter BBB permeability (Hoffman et al. 2009). Metabolism of ketones leads to an inhibition of glycolysis and decrease in glucose utilization by the brain. Elevated brain glucose and ketone levels may also cause oxidative stress resulting in disruption of membrane structures. The combination of microvascular effects and membrane disruption could possibly contribute to the development of cerebral edema. Detection of lactate indicate anaerobic cerebral metabolism and causes cerebral hypofusion and play a role in the pathogenesis of cerebral edema. Diabetic ketoacidosis leads to cerebral edema that arises in ∼1 % of episodes and is a complication that frequently causes irreversible brain damage and death. Cerebral edema particularly increases the intracranial pressure and causes neurological collapse (Yuen et al. 2008). Cerebral edema occurs rarely in patients older than age 20 years and mostly occurs in children. An increase in taurine and myoinositol levels within the brain in children with diabetic ketoacidosis has also been suggested (Vavilala et al. 2010). Many theories have been proposed to explain the development of cerebral edema in diabetic ketoacidosis but the exact etiology is complex and multifactorial.
Several markers of vascular inflammation have been shown to be influenced by the presence of ketosis. It has been reported that acetoacetate, but not β-hydroxybutyrate, increases lipid peroxidation as well as lowering GSH levels. It has also been reported that acetoacetate increases TNF-α and IL-6 secretion in hyperketonemic diabetic patients (Kitabchi et al. 2004). Other reports have shown that chronic exposure to β-hydroxybutyrate can impair insulin action. These studies also showed an increase in ROS production and inhibition of the AMPK/p38 MAPK signaling pathway (Hoffman et al. 2003). These studies also indicated that high levels of ketone bodies can increase cellular oxidative stress, which may contribute to the development of the insulin resistance seen in both types of diabetes.

2.7 CURRENT TREATMENT FOR DIABETIC ENCEPHALOPATHY

Various studies have suggested a number of therapeutic strategies to combat diabetes related CNS deficits. Some of the areas are discussed as below:-

(1) Research into AGEs has been conducted with the goal for preventing CNS related diabetic complications. The goals were to prevent or slow AGEs formation and break the AGE cross-links between proteins and possibly reverse the damage. The potential importance of AGEs in the pathogenesis of diabetic complications has been shown by two AGE inhibitors- (i) Pyridoxamine that inhibit glycation reactions and the formation of AGEs (Voziyan & Hudson 2005) and (ii) Pimagedine, which inhibits AGEs formation. These molecules did not demonstrate a statistically significant beneficial effect on the progression of CNS complication associated with type 1 diabetes (Bolton et al. 2004).

(2) Experimental evidence implicates PARP as a causative factor in the pathogenesis of diabetes and diabetic complications, such as acute endothelial dysfunction (Szabo 2005). PARP catalyzes the transfer of ADP-ribose units from the substrate NAD+ to acceptor proteins, biosynthesizing polyanionic poly(ADP-ribose) polymers. Hyperglycemia-induced ROS overproduction leads to DNA strand breakage and subsequent PARP activation. It slows the rate of glycolysis, electron transport and ATP formation. PARP also promotes the activation of various pro-inflammatory signal transduction pathways. The therapeutic potential of PARP inhibition in the prevention or reversal of diabetic complications has been the subject of intensive research work and works on the downstream effectors (Woon & Threadgill 2005).

(3) Antioxidant therapy has been of great interest as a way to combat oxidative stress in diabetic patients over the past decade. Classical antioxidants such as vitamins E and C do not
seem to be helpful among all diabetic patients (Golbidi et al. 2011). Although some antioxidants like N-acetyl cysteine, Curcumin, Catalpol, Aucubin have been successful in preventing CNS complications associated with diabetes but they have shown beneficial effect at very high doses. Therefore, new insights into mechanisms leading to oxidative stress conditions may provide new antioxidant discoveries.

2.8 GSNO AS A NEUROPROTECTANT

GSNO is a physiological metabolite of glutathione GSH and NO (Schrammel et al. 2003) (Fig. 15). It is involved in several pharmacological activities and cellular signalling (Nakamura et al. 1991). GSNO is present in micromolar concentrations in the rat brain (Kluge et al. 1997). It is several-fold more potent than GSH against oxidative stress caused by ONOO$^-$ (Rauhala et al. 1998). NO donors especially GSNO has been shown to be highly potent antioxidant against oxidative stress caused by ONOO$^-$ and lipid peroxidation. GSNO compared to SNAP may be related with the release of GSSG leading to increase in GSH and also glutathionylation by GSNO of target proteins. Unlike other classes of NO donor, GSNO is a stable compound and does not decompose spontaneously; it requires additional agents or enzymes, including GSNOR or the thioredoxin system (Zeng et al. 2001), for its metabolism. GSNO could transduce the signal by trans-nitrosylating target proteins and GSNOR would down-regulate signaling by removing GSNO (Liu et al. 2001). Although the specificity of GSNOR clearly implicates GSNO levels as an important modulator of signaling, it should be noted that GSNO can breakdown to release NO, as well as transfer the NO adduct to other sulfhydryls (Bryan 2006).

![Figure 15: Structure of S-Nitrosoglutathione.](image)

GSNO may function as stabilizer, reserve and carrier of NO. NO can activate guanylate cyclase, leading to cGMP-mediated up-regulation of thioredoxin and thioredoxin peroxidase enzymes. Moreover, up-regulation of thioredoxin system leads to removal of H$_2$O$_2$, repair of oxidized proteins and up-regulation of Mn-superoxide dismutase (Mn-SOD) enzyme, all of which resist oxidative stress and injury. NO-mediated up-regulation of
thioredoxin, Bcl-2 and MnSOD leads to the cGMP-dependent antiapoptotic and antioxidative action that enhances cell viability and survival (Andoh et al. 2002). S-nitrosylation of thioredoxin also increased the activity of this protective enzyme. At moderate concentrations, NO and GSNO act like a free radical scavenger which are 50 to 100 times more potent than the classic antioxidant glutathione. It has been recently highlighted that homeostasis in signaling pathways involving S-nitrosylation may be crucial (Foster et al. 2003). In addition, GSNO may directly S-nitrosylate proteins (R-S-NO) and modulate their activity, inhibiting caspase-3 and apoptosis signal regulating kinase-1 (ASK-1) and activating thioredoxin. Lipid peroxidation, which goes hand-in-hand with inflammation in injured brain tissue was decreased by the treatment of GSNO in a rat model of experimental stroke (Rauhala et al. 1998).

GSNO also reduces the frequency of embolic signals and can reverse acute vasoconstriction (Kaposzta et al. 2002). Administration of GSNO has been shown to suppress iNOS induction and enhance eNOS expression in pedicle vessels, resulting in blood perfusion and a higher flap survival after ischemia/reperfusion (Kuo et al. 2004). In animals, GSNO treatment has been found to lower inflammation and secondary injury in the brain (Khan et al. 2006). The anti-inflammatory activity of GSNO in down regulating iNOS is mediated by the inhibition of NF-κB activation in rat primary astrocytes and microglial cell lines (Khan et al. 2005). GSNO improved neurobehavioral functions via reducing apoptotic cell death, inflammation and BBB leakage. Other GSNO-mediated effects were decreased contusion volume, improved tissue structure and reduced edema as being implicated in diabetes as well. The treatment with GSNO was also found to enhance the expression of ZO-1 and occludin and decrease the expression of MMP-9, indicating its potential for BBB protection thus improving neurobehavioral functions in rat model of traumatic brain injury (Khan et al. 2009). GSNO mainly functions via nitrosylation of protein thiols in health and disease. It is an endogenous nitrosylating agent and its homeostasis is maintained in blood and tissues (Martinez-Ruiz et al. 2005). GSNO reduces not only neuronal cell death and glial cell inflammation but also reduces endothelial cell activation evaluated as the expression of ICAM-1 and E-selectin, indicating the potential of GSNO therapy for the protection of BBB (Khan et al. 2006). Activation of endothelial cells via increased expression of ICAM-1 is directly involved in BBB disruption and inactivation of ICAM-1 provides protection in BBB (Bowes et al. 1993). GSNO decreased Evans blue extravasation (a measure of BBB leakage), suggesting that GSNO protects BBB integrity. Expression of both ICAM-1 and MMP-9 are regulated by NF-κB; therefore, GSNO may reduce the expression of both MMP-9 and
ICAM-1 via inhibition of NF-κB through nitrosylation of the P65 subunit of NF-κB as reported earlier (Prasad et al. 2007).

GSNO has also been used to protect endothelial tight junctions and therefore keeps BBB intact (Khan et al. 2009). Interestingly, ZO-1 has been reported to contain a transnitrosylation consensus motif at cysteine amino acid residue 1718. However, whether 1718 cysteine in ZO-1 is nitrosylated and the consequent effect of nitrosylation is not known. Blood leakage into the parenchyma through the compromised BBB contributes to inflammation, disrupting cellular defense mechanisms.

![Diagram](image)

**Figure 16: Neuroprotective effects of S-Nitrosoglutathione.**

All these pathophysiologies have also been implicated in diabetes induced hyperglycemia that attributed us to use GSNO as a novel therapeutic agent to improve cognitive decline in diabetic experimental animals.