2.1. DIABETES MELLITUS

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolisms, mainly due to deficiency of insulin secretion and/or insulin action; characterized by hyperglycemia; if not controlled, may lead to long term complications, like Neuropathy, Retinopathy and Nephropathy.

2.1.1. Historical Background

Modern clinicians can still identify with the eloquence of the sentiments expressed almost 2000 years ago by the second-century Greek physician Aretaeus of Cappadocia when he coined the term *diabetes* to describe an affliction with no known treatment. (MacFarlane I.A. et al, 1997). By the end of the first millennium AD, writers in India, China, Japan and Middle East had described most of the salient features of the disease. They had noted that the polyuria of diabetes was sugary. Two forms of the disease were recognized, one afflicting older, overweight people and the other, more rapidly fatal, developing in younger, thin patients. In the 17th century, the English physician Thomas Willis arguably first noted the onset of our current epidemic is diabetes. The roots of some of the trendiest lifestyle prescriptions for diabetes management were well described by the end of the 18th century as John Rollo attempted to treat patients with a diet rich in meats and restricted in carbohydrates. Through the 19th and 20th centuries, dramatic advances have been made regarding our understanding of the etiology of the disorder, most importantly the identification of the role of the beta cell and insulin action. (MacFarlane I.A. et al, 1997).
2.1.2. Epidemiology:

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate (Huizinga MM. et al 2006). Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people. It is important to note that the rise in prevalence is seen in all six inhabited continents of the globe (Wild S. et al, 2004). According to the Global report on diabetes by World Health Organization, Geneva, 2016, the number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014. The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014. Diabetes prevalence has been rising more rapidly in middle- and low-income countries. In 2012, an estimated 1.5 million deaths were directly caused by diabetes and another 2.2 million deaths were attributable to high blood glucose. WHO projects that diabetes will be the 7th leading cause of death in 2030. According to IDF Atlas 2015 now China ranks first with 109.6 million followed by India 69.3 millions of diabetes cases though 46.5% of adults with diabetes are still undiagnosed.

Now diabetes as epidemic is more pronounced in India as the World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2000 (Huizinga MM. et al 2006). The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025 (Sicree R. et al, 2006). The so called “Asian Indian Phenotype” refers to certain unique clinical and biochemical abnormalities in Indians which include increased insulin resistance, greater abdominal adiposity i.e., higher waist circumference despite lower body mass index, lower adiponectin and higher high sensitive C-reactive protein levels. This phenotype makes Asian Indians more prone to diabetes and premature coronary artery disease. At least a
part of this is due to genetic factors. However, the primary driver of the epidemic of diabetes is the rapid epidemiological transition associated with changes in dietary patterns and decreased physical activity as evident from the higher prevalence of diabetes in the urban population.

The first national study on the prevalence of type 2 diabetes in India was done between 1972 and 1975 by the Indian Council Medical Research (ICMR, New Delhi)( Ahuja MMS. Et al, 1979) . Screening was done in about 35,000 individuals above 14 yr of age, using 50 g glucose load. Capillary blood glucose level >170 mg/dl was used to diagnose diabetes. The prevalence was 2.1 per cent in urban population and 1.5 per cent in the rural population while in those above 40 yr of age, the prevalence was 5 per cent in urban and 2.8 per cent in rural areas. Subsequent studies showed a rising trend in the prevalence of diabetes across different parts of India. In 1988, a study done in a small township in South India reported a prevalence of 5 per cent (Ramachandran A. et al,1988) . A national rural diabetes survey was done between 1989 and 1991 in different parts of the country in selected rural populations (Sridhar GR. Et al , 2002) . This study which used the 1985 WHO criteria to diagnose diabetes, reported a crude prevalence of 2.8 percent(Sridhar GR. Et al , 2002) . The Eluru survey which looked at the prevalence of known diabetes in four villages in Andhra Pradesh showed a prevalence of 1.5 per cent. The prevalence of known diabetes was 6.1 per cent in individuals aged above 40 yr which was unexpectedly high at that time for a rural area with low socio-economic status and decreased health awareness(Rao PV. Et al, 1989) . A study done in 1988 in Chennai reported a prevalence of 8.2 per cent in the urban and 2.4 per cent in the rural areas(Ramachandran A. et al, 1992) . A subsequent study in the same urban area done after five years showed an age standardized prevalence of 11.6 per cent indicating a rising trend in prevalence of diabetes (Ramachandran A. et al, 1997) . A very high prevalence of 16.3 per cent was reported in Thiruvananthapuram in
Kerala State in the year 1999 (Raman Kutty V. et al, 1999). In the same year, a prevalence of 8.2 per cent was reported from Guwahati (Shah SK. et al, 1999). A cross-sectional population survey was done in the Kashmir valley in 2000 and the prevalence of ‘known diabetes’ among adults aged >40 yr was found to be 1.9 per cent (Zargar AH. et al, 2000). An oral glucose tolerance test was done using capillary glucose and diabetes was defined using the WHO criteria (Alberti KG. et al, 1998). The study reported that the age standardized prevalence of type 2 diabetes was 12.1 per cent. This study also revealed that the prevalence in the southern part of India to be higher-13.5 per cent in Chennai, 12.4 per cent in Bangalore, and 16.6 per cent in Hyderabad; compared to eastern India (Kolkata) 11.7 per cent; northern India (New Delhi), 11.6 per cent; and western India (Mumbai), 9.3 per cent. The study also suggested that there was a large pool of subjects with impaired glucose tolerance (IGT), 14 per cent with a high risk of conversion to diabetes. A study done in western India showed age standardized prevalence of 8.6 per cent in urban population (Gupta A. et al, 2003). A more recent study reported a high prevalence (9.3%) in rural Maharashtra (Deo SS. et al, 2006). The Amrita Diabetes and Endocrine Population Survey (ADEPS) (Menon VU. et al, 2006), a community based cross-sectional survey done in urban areas of Ernakulam district in Kerala has revealed a very high prevalence of 19.5 per cent.

2.1.3. Type of Diabetes

In 1980, the World Health Organization (WHO) proposed a classification of diabetes mellitus based on the recommendation of the US National Diabetes Data Group. In 1997, the American Diabetes Association (ADA) again reclassified diabetes, revised the diagnostic criteria. The 1997 ADA classification of diabetes, subsequently endorsed by the WHO, recognizes four main categories:
I. Type 1 diabetes: Previously known as ‘insulin-dependent’ or ‘juvenile onset’ diabetes-characterized by selective islet β-cell destruction, absolute insulin deficiency and reliance on exogenous insulin to preserve life.

II. Type 2 diabetes: Previously known as ‘non-insulin dependent’ or ‘maturity-onset’ diabetes- occurs mostly after the age of 30.

III. Gestational diabetes: When diabetes mellitus is diagnosed during pregnancy, it is referred to as gestational diabetes. Gestational diabetes usually resolves post-partum.

IV. Other specific forms: Diabetes may be secondary to a variety of diverse conditions, including specific genetic or acquired syndromes and the use of certain drugs.

A. Genetic defects of β-cell function, eg MODY syndrome [Maturity Onset Diabetes of the Young due to specific genetic defects of glucokinase or hepatic nuclear factors]

B. Genetic defects in Insulin action, eg Leprechaunism

C. Diseases of the exocrine pancreas, eg Pancreatitis.

D. Secondary to endocrinopathies, eg Acromegaly, Cushing’s Syndrome.

E. Drug- or Chemical- induced, eg by Glucocorticoids

F. Infections, eg Congenital Rubella

G. Uncommon forms of Immune-mediated diabetes, eg Anti-insulin receptor antibodies

H. Other genetic syndromes associated with diabetes, eg Down’s syndrome.

2.1.4. Clinical Features of Diabetes

The presenting clinical features of Type 2 diabetes range from surprisingly few symptoms in some patients to dramatic and life-threatening hyperglycaemic emergency of hyperosmolar
non-ketotic coma or DKA (Diabetes Ketoacidosis). So, although classic osmotic syndromes are the rule in type 2 diabetes, a high index of clinical suspicion must be maintained if asymptomatic cases are to be identified.

Presenting features of Type 2 Diabetes

<table>
<thead>
<tr>
<th>Minimal</th>
<th>Asymptomatic patients are identified by screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmotic symptoms</td>
<td>Thirst</td>
</tr>
<tr>
<td></td>
<td>Polyuria</td>
</tr>
<tr>
<td></td>
<td>Nocturia</td>
</tr>
<tr>
<td></td>
<td>Blurred Vision</td>
</tr>
<tr>
<td>Infection</td>
<td>Recurrent fungal infections (eg genital candidiasis)</td>
</tr>
<tr>
<td></td>
<td>Recurrent bacterial infections (eg Urinary Tract Infection)</td>
</tr>
<tr>
<td>Macrovascular Complications</td>
<td>Coronary Artery Disease</td>
</tr>
<tr>
<td></td>
<td>Peripheral Vascular Disease</td>
</tr>
<tr>
<td>Microvascular Complications</td>
<td>Nephropathy</td>
</tr>
<tr>
<td></td>
<td>Neuropathy</td>
</tr>
<tr>
<td></td>
<td>Retinopathy</td>
</tr>
<tr>
<td>Associated Conditions</td>
<td>Glaucoma</td>
</tr>
<tr>
<td></td>
<td>Cataract</td>
</tr>
</tbody>
</table>
2.1.5. Pathophysiology of Type 2 Diabetes

Pathophysiology of type 2 diabetes mellitus results from complex interplay of insulin resistance and beta cell dysfunction and adipokines. Patients with type 2 diabetes share a pathophysiology that involves the pancreatic beta cells, the liver and peripheral target tissues, namely skeletal muscle and adipose tissue. A variable degree of beta cell dysfunction is present in these patients in addition to hepatic insulin resistance, resulting in glucose overproduction. Glucose uptake into muscle cells requires insulin binding to cell-surface receptors and activation of insulin signaling cascade, which in turn facilitates the movement of glucose across the cell-membrane; ultimately the glucose is stored as glycogen or is used as an energy source via glycolysis to lactate or mitochondrial oxidation.

Maintenance of normal glucose levels depends upon a closed feedback loop between the circulating glucose level and the pancreatic hormones, insulin and glucagon. In the fasting state, glucose is largely produced by the liver via glycogen breakdown and gluconeogenesis. Approximately 70% to 80% of the glucose produced by the liver is used by the brain, and by
other insulin-sensitive tissues, such as gastrointestinal tract and erythrocytes. Insulin (from the pancreatic beta cell) exerts an inhibitory effect on hepatic glucose production. In contrast, glucagon (from the pancreatic alpha cell) stimulates hepatic glucose production. If peripheral insulin sensitivity changes, this would result in a change in serum glucose, which in turn result in modulation of insulin and glucagon in order to maintain glucose level. Complete adaptation actually doesn’t occur when a new higher steady-state glucose level is reached.

It is well accepted that the pathophysiology of type 2 diabetes involves progressive beta cell dysfunction. Conceivably, this is because of a decrease in beta cell mass, beta cell dysfunction, or to both abnormalities. Frequency of beta cell neogenesis or beta cell apoptosis can be calculated with the equation:

Frequency of beta cell replication or apoptosis = \( \frac{\text{cell or islet}}{\% \text{beta cell area}} \).

Of late the role of the incretin pathway is being emphasized in the pathogenesis of type 2 diabetes. Nutrient intake stimulates the secretion of the gastrointestinal incretin hormones, glucagon like peptide 1 (GLP 1) and glucose-dependent insulino tropic polypeptide (GIP), which exert glucose-dependent insulinotropic effect and assist pancreatic insulin and glucagon in maintaining glucose homeostasis. GLP 1 also suppresses glucose-dependent glucagon secretion, slows gastric emptying, increase satiety and reduce food intake. An impaired incretin system, characterized by markedly reduced GLP 1 concentration occurs in individuals with type 2 diabetes. Administration of GLP 1 improves glycemic control. A growing understanding of the role of incretin hormones in T2D may further clarify the application of incretin based treatment strategies.
2.1.6. Diagnosis

A blood or plasma glucose measurement is the essential investigation in the diagnosis of diabetes. An appropriate sample of venous plasma is collected in fluoride oxalate to inhibit glycolysis.

The revised diagnostic criteria for diabetes (according to the ADA,1997) are as follows

- Fasting plasma glucose $\geq 7.0$ mmol/l (126 mg/dl)
- Post prandial plasma glucose $\geq 11.0$ mmol/l (200 mg/dl) [2 hrs after 75 gm glucose taken orally]

More recently HbA1c assay is standardized to diagnose diabetes above the value 6.5%.

2.1.7. Complications of Diabetes

People with diabetes have an increased risk of developing a number of serious health problems. Consistently high blood glucose levels can lead to serious diseases affecting the heart and blood vessels, eyes, kidneys, nerves and teeth. Long-term complications of diabetes develop gradually. Eventually, diabetes complications may be disabling or even life-threatening. These complications are categorized as follows:

<table>
<thead>
<tr>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic ketoacidosis</td>
<td>Macrovascular</td>
</tr>
<tr>
<td>Hyperosmolar non-ketotic</td>
<td>Microvascular</td>
</tr>
<tr>
<td>coma</td>
<td>Coronary artery disease (CAD)</td>
</tr>
<tr>
<td></td>
<td>Peripheral vascular disease (PVD)</td>
</tr>
<tr>
<td></td>
<td>Cerebrovascular disease</td>
</tr>
<tr>
<td></td>
<td>Diabetic Neuropathy</td>
</tr>
<tr>
<td></td>
<td>Diabetic Retinopathy</td>
</tr>
<tr>
<td></td>
<td>Diabetic Nephropathy</td>
</tr>
</tbody>
</table>
2.2. Diabetic Neuropathy

Diabetic neuropathies are nerve disorders associated with diabetes mellitus. These conditions are thought to result from diabetic microvascular injury involving small blood vessels that supply nerves in addition to macrovascular conditions that can culminate in diabetic neuropathy. Relatively common conditions which may be associated with diabetic neuropathy include third nerve palsy; mononeuropathy; mononeuropathy multiplex; diabetic amyotrophy; a painful polyneuropathy; autonomic neuropathy; and thoracoabdominal neuropathy. Diabetic neuropathy affects a combination of distinct regions of the nervous system and may be asymptomatic, remaining undetected while causing extensive damages. Commonest clinical manifestation is bilateral symmetrical peripheral neuropathy. The major complication of somatic neuropathy is foot ulceration which often precedes gangrene and limb loss.

2.2.1. Epidemiology

Diabetic neuropathy encompasses a wide, heterogeneous group of clinical and subclinical syndromes. It is one of the major long term complications associated with diabetes that can cause considerable morbidity and mortality( Vinik AI et al,1994). Although the diagnosis of diabetic neuropathy can vary significantly, the prevalence ranges from 5%-45% in the United States and other developed countries (EURODIAB IDDM et al,1994; Holzer et al,1998; Caputo GM et al,1994; Young MJ et al,1993; Dyck PJ et al, 1993; Pirart et al,1977). It is estimated that the prevalence of neuropathy in diabetes patients is approximately 20%. In the DCCT (Diabetes Control and Complications Trial, 1995) study, the annual incidence of neuropathy was 2% per year. Globally diabetic neuropathy affects approximately 132 million people as of 2010 (1.9% of the population). (Vos T, 2012 ).
2.2.2. Classification

Diabetic neuropathy affects the sensory, automatic, and motor neurons of the peripheral nervous system; therefore, it is classified according to the category of nerve fibers involved.

a) Mononeuropathy

b) Mononeuropathy multiplex

c) Diabetic Amyotrophy

d) Autonomic Neuropathy

e) Bilateral Sensorimotor distal Polyneuropathy affecting limbs- commonest clinical type.

2.2.3. Clinical Features of Diabetic Neuropathy

- *Absent ankle jerks* - an early feature, but age-related loss is also common in the elderly.

- *Diminised vibration sense* - a hammer is used to the terminal phalanx(big toe). If sensation is absent, the hammer is moved progressively to the medial malleolus, then along the upper tibia.

- *Warm and dry skin* - with fissuring due to the local sympathetic denervation.

- *Reduction in other sensory modalities* – position, light touch, pain and temperature may accompany or follow loss of vibration sensation.

- *Dilated superficial veins* – and sometimes bounding pedal pulses due to vascular shunting.
2.2.4. Pathogenesis

Although the exact mechanism of diabetic neuropathy has yet to be fully understood, there are several pathogenic mechanisms involved in diabetic neuropathy.

*Hyperglycemia:* Chronic hyperglycemia is the main initiator of neurovascular damage. Patients who have poor glycemic control develop complications (Gaede P et al, 2003). In addition to low insulin and C-peptide, patients with diabetes have elevated blood glucose, which may lead to nerve damage (Hale PJ et al, 1987). The nerve damage may be a result of alterations in neural ion transport. Hyperglycemia leads to glycosylation of proteins in newly formed cells. Increased nonenzymatic glycosylation of proteins in nerve cells damages nerves by preventing the nerve cells from sending and receiving signals from stimuli.

- *Advanced Glycation End Products:* An intracellular increase in glucose results in the formation of AGEs, which, along with other factors, may initiate an inflammatory cascade (King RH et al, 2001). AGEs are increased during aging as well as diabetes by autooxidation of glucose and its binding to proteins. The protein binding is initially reversible, and then irreversible, modifying the proteins and their structures with glucose (King GL et al, 1996). Formation of AGE damages cells by altering cell surface interactions, which activates intracellular signaling pathways to alter endothelial functions (Chibber R et al, 1999; Brownlee M et al, 2000). AGE can bind to cell surface receptor proteins called receptor for AGE (RAGE). These receptors cause cellular dysfunction through a cascade of events. The presence of AGEs and RAGE in patients with diabetes suggests that these metabolic end products are a part of etiology of neuropathy.
• **Aldose Reductase (Polyol) Pathway:** In patients with diabetes, excess glucose not metabolized by glycolysis enters the polyol pathway. There is increased flux through the polyol pathway, resulting in elevated nerve levels of glucose, fructose, and sorbitol, possibly due to enhanced aldose reductase and reduced sorbitol dehydrogenase (Oates PJ et al, 2002). Sorbitol accumulation leads to cellular and osmotic stress. Thus it can alter enzyme activities which contribute to diabetic microvascular complications.

• **Oxidative Stress:** Each of these pathways leads to oxidative stress and formation of reactive oxygen species (ROS). During hyperglycemia, oxidative stress is increased by superoxides, which occur at abnormally high levels due to increased glycolysis and lypolysis with impaired mitochondrial activity. Superoxide is metabolized to H$_2$O$_2$ by Superoxide dismutase. H$_2$O$_2$ is freely diffusible and reacts with iron to form OH radicals that damage lipids. The resultant lipid peroxides cause cell death.

### 2.2.5. Diagnosis

Diabetic neuropathy is usually diagnosed based on patient’s symptoms, medical history and a physical exam. During the exam, muscle strength and tone, tendon reflexes, and sensitivity to touch, temperature and vibration are checked.

• **Nerve Conduction Velocity studies.** This test measures how quickly the nerves in arms and legs conduct electrical signals. An NCV test is done using electrodes that are patched onto the skin. These are placed along a nerve pathway—one at the top of the leg and one further down. A tiny electrical current stimulates the nerve at one electrode, and then the second electrode captures the signal as it passes down the nerve. The test measures how long it took the signal to travel down the nerve.
2.3. Diabetic Retinopathy

Diabetic retinopathy is a condition that occurs in people who have diabetes. It causes progressive damage to the retina, the light-sensitive lining at the back of the eye. Diabetic retinopathy is a serious sight-threatening complication of diabetes. The disease is characterized by too much sugar in the blood, which can cause damage throughout the body, including the eyes. Over time, diabetes damages the blood vessels in the retina. Diabetic retinopathy occurs when these tiny blood vessels leak blood and other fluids. This causes the retinal tissue to swell, resulting in cloudy or blurred vision. The condition usually affects both eyes. When people with diabetes experience long periods of high blood sugar, fluid can accumulate in the lens inside the eye that controls focusing. This changes the curvature of the lens, leading to blurred vision. The longer a person has diabetes, the more likely they will develop diabetic retinopathy. If left untreated, diabetic retinopathy can cause blindness. However, once blood sugar levels are controlled, blurred distance vision will improve. Patients with diabetes who can better control their blood sugar levels will slow the onset and progression of diabetic retinopathy.

2.3.1. Epidemiology

The incidence of blindness is 25 times higher in patients with diabetes than in the general population (Klein R et al, 1985). The duration of the diabetes is the most important predictor of diabetic retinopathy. Patients with tight control had a 76% reduction in the rate of development of any retinopathy and a 54% reduction in progression of established retinopathy as compared with the conventional treatment group. For advanced retinopathy, however, even the most rigorous control of blood glucose may not prevent progression (DCCT, 1993).

There are approximately 93 million people with DR worldwide. Diabetic retinopathy (DR) is the leading cause of blindness among working-aged adults around the world (Klein BE, 2007).
M.W. Knuiman (Knuiman MW et al, 1986) reported prevalence of retinopathy at 28% in Perth, Western Australia. Caird et al (Caird F.I. et al, 1968) found a prevalence rate of 36.8% NPDR in a survey which involved 4076 diabetic patients with over ten years duration of diabetes. DR prevalence estimates among individuals with both diagnosed and undiagnosed diabetes, with rates ranging from 17.6% in a study in India (Rema M et al, 2005) to 33.2% in a U.S. study (Wong TY et al, 2006). According to a study the prevalence of diabetes is rising notably in Asian countries such as India and China (Shaw JE et al, 2010; , Yang W et al,2010). Ramchandran et al 1999 (Ramachandran et al, 1999) who observed retinopathy in 714 i.e. 23.7% cases out of 3010 patients of type 2 diabetes. In other studies the prevalence of retinopathy at diagnosis varies from 20- 30%. The reasons for these differences are not clear.

According to the latest World Health Organization (WHO) report, India has 31.7 million diabetic subjects, and the number is expected to increase to a staggering 79.4 million by 2030.( Wild S et al, 2004).

2.3.2. Classification

Chronically high blood sugar from diabetes is associated with damage to the tiny blood vessels in the retina , leading to diabetic retinopathy. Diabetic retinopathy is classified into two main categories:

1. **Non-proliferative diabetic retinopathy** (NPDR) is the early stage of the disease in which symptoms are mild or nonexistent. In NPDR, the blood vessels in the retina are weakened. Tiny bulges in the blood vessels, called microaneurysms, may leak fluid into the retina. This leakage lead to swelling of the macula.
2. **Proliferative diabetic retinopathy** (PDR): At this advanced stage, growth factors secreted by the retina trigger the proliferation of new blood vessels, which grow along the inside surface of the retina and into the vitreous gel, the fluid that fills the eye. The new blood vessels are fragile, which makes them more likely to leak and bleed. Accompanying scar tissues contract and cause retinal detachment—the pulling away of the retina from underlying tissue. Retinal detachment can lead to permanent vision loss.

2.3.3. **Clinical Features of Diabetic Retinopathy**

- Gradual loss of vision, suggestive of the development of maculopathy or cataract.
- Sudden, painless, loss of vision due to vitreous haemorrhage. Retinal arterial and venous thrombosis may occur in patients in diabetes.
- Appearance of ‘floaters’, possibly due to small or recurrent vitreous haemorrhages.
- Chronic pain and redness.
2.3.4. Pathogenesis

The mechanism by which lack of glycemic control predisposes to vascular disease is incompletely understood. Chronic hyperglycemia is thought to be the primary cause of diabetic retinopathy (Frank RN, 1991). Proposed contributing factors are hyperglycemia, advanced glycation end products, aldose reductase pathway etc.

- **Hyperglycemia:** Chronic hyperglycemia is thought to be the primary cause of diabetic retinopathy. Intensive therapy reduced the incidence of new cases of retinopathy and the reduction was directly related to the degree of glycemic control as estimated from hemoglobin A1c (HbA1c) values; progressive retinopathy was uncommon in patients with HbA1c values lower than 7% (Frank RN, 1991).

- **Advanced Glycation End Products:** Chronic hyperglycemia results in glycosylation of serum and tissue proteins leading to the formation of advanced glycation end products (Vlassara H, 1996; Brownlee M, 1994). Circulating AGE concentrations are increased in diabetic patients. They can increase vascular permeability, promote the influx of mononuclear cells, and stimulate cell proliferation (Vlassara H, 1996; Brownlee M, 1994). The net effect is tissue accumulation of AGEs, which can contribute to the microvascular complications (Makita Z et al, 1991).

- **Aldose Reductase:** Under hyperglycemic conditions, glucose that enters cells is partly metabolized to sorbitol via the rate-limiting enzyme aldose reductase (the polyol pathway). Sorbitol is then metabolized to fructose, a process that is relatively slow. Sorbitol accumulation within the crystalline lens increases with chronic hyperglycemia (Frank RN, 1994; Peterson CA et al, 1992). It leads to rise in intracellular osmolarity, which causes absorption of water into the cells and cellular swelling. The resultant damage to lens
epithelial cells, which have a high concentration of aldose reductase, is responsible for diabetic cataract formation. Aldose reductase is also found in high concentration in retinal pericytes. In this tissue the polyol pathway can result in alteration of cellular metabolism, leading to basement membrane thickening and other diabetic complications (Peterson CA et al, 1992).

2.3.5. Diagnosis

In patients with diabetes mellitus, regular *Funduscopic examination* are important to screen for diabetic retinopathy as visual loss due to diabetes can be prevented by retinal laser treatment if retinopathy is spotted early.

**Funduscopic examination**: It consists exclusively of inspection. One looks through the *ophthalmoscope*, which is simply a light with various optical modifications, including lenses. The ophthalmoscope illuminates the retina through the normal iris defect that is the pupil. Light rays forming the image of the retina re-emerge through the pupil. The viewing aperture (window) of the ophthalmoscope contains a lens that modifies light rays to assist the user. In the procedure, one looks at structures lying in the innermost aspect of the globe, collectively known as the *eyegrounds*: retina, retinal blood vessels, optic nerve head (disk).

*Funduscopic examination* recognizes the pre-proliferative lesions. Thus it prevents its occurrence, and its typical sequelae of retinal and vitreous hemorrhage, and permanent blindness.

2.4. PREDISPOSING FACTORS:

The most common use of the term “predisposing factors” in the field of public health has been in the context of L.W. Green’s PRECEDE-PROCEED model of community health promotion planning and evaluation (Green et al., 1999). “Predisposing factors” are defined in this models as
factors that exert their effects prior to a behavior occurring, by increasing or decreasing a person or population’s motivation to undertake that particular behavior.

There are different types of Predisposing factors exist, those are:

- Lifestyle Factors
- Biochemical Factors
- Genetic Factors
- Environmental Factors
- Effect of Enzymes

2.4.1. LIFESTYLE FACTORS:

2.4.2. AGE:

The prevalence of type 2 diabetes increases with age; up to 20% of those over 80 years old develop diabetes. The ageing population of many societies has contributed substantially to the overall increase in the number of patients with diabetes. Glucose tolerance decreases with age, but the extent of the natural deterioration in insulin sensitivity with age remains uncertain. The weight gain that commonly occurs between the fourth and seventh decades of life creates its own state of insulin resistance, particularly if this adiposity is of central (abdominal) distribution.

Recent years have witnessed the emergence of type 2 diabetes in younger groups, including children, adolescents and young adults. This trend is of particular concern, since the clinical course of type 2 diabetes and development of long-term tissue complications are largely determined by the duration and the degree of hyperglycemia.
2.4.3. OBESITY:

Most patients with type 2 diabetes are obese, and the global epidemic of obesity largely explains the dramatic increase in the incidence and prevalence of type 2 diabetes over the past 20 years. Currently, over a third (34%) of U.S. adults are obese (defined as BMI >30 kg/m$^2$), and over 11% of people aged ≥20 years have diabetes (Centers for Disease Control and Prevention, 2011), a prevalence projected to increase to 21% by 2050 (Boyle JP et al., 2010).

The influence of obesity on type 2 diabetes risk is determined not only by the degree of obesity but also by where fat accumulates. Increased upper body fat including visceral adiposity, as reflected in increased abdominal girth or waist-to-hip ratio, is associated with the metabolic syndrome, type 2 diabetes, and cardiovascular disease (Björntorp P, 1991). At least three distinct mechanisms have been proposed to link obesity to insulin resistance and predispose to type 2 diabetes:

1) increased production of adipokines/cytokines, including tumor necrosis factor-α, resistin, and retinol-binding protein 4, that contribute to insulin resistance as well as reduced levels of adiponectin (Deng Y et al., 2010);

2) ectopic fat deposition, particularly in the liver and perhaps also in skeletal muscle, and the dysmetabolic sequelae (Larson-Meyer DE et al., 2011); and

3) mitochondrial dysfunction, evident by decreased mitochondrial mass and/or function (Bournat JC et al., 2010).
2.4.4. FAMILY HISTORY OF DIABETES

<table>
<thead>
<tr>
<th>Family History</th>
<th>Risk Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>One parent with T2D</td>
<td>1 in 13</td>
</tr>
<tr>
<td>Both parents with T2D</td>
<td>1 in 2</td>
</tr>
<tr>
<td>Identical twin with diabetes</td>
<td>3 in 4</td>
</tr>
</tbody>
</table>

Data from American Diabetes Assoc patient education information

Family history of diabetes is indeed a powerful independent risk factor for the disease. Previous studies have indicated that, people without a family history of diabetes, versus those who have a family history of diabetes are two to six times as likely to have type 2 diabetes (Harrison TA et al. 2003).

A recent study based on National Health and Nutrition Examination Survey (NHANES) data found that, 1) family history of diabetes was significantly and independently associated with diabetes in U.S. adults (based on self-reports) and 2) the strength of the association was related to the type and number of relatives involved (Annis AM et al., 2005). Recent studies have shown the graded and independent contribution of a positive family history to the increasing risk for diabetes in the U.S. population (Hariri S et al., 2006).

2.5. BIOCHEMICAL FACTORS

2.5.1. BLOOD SUGAR PROFILE

Many literature advocate the importance of early diagnosis in order to reduce diabetes complications (Deedwania PC., et al, 2005). It is estimated that about one third of people with type 2 diabetes might be undiagnosed until the complications are developed (American Diabetes
Glycemic control is the most important aspect in management of diabetes mellitus. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Vinod Mahato R. et al, 2011). Many large randomized clinical trials and observational studies in type 1 and 2 diabetes have clearly shown that achieving glycemic control or reducing hyperglycemia significantly decrease the microvascular and macrovascular complications of diabetes mellitus (DM) (UK Prospective Diabetes Study (UKPDS) 1998). Therefore, establishing efficient screening programs to detect people with undiagnosed diabetes is important. Control of plasma glucose in patients with diabetes can be assessed by measurement of fasting plasma glucose (FPG), postprandial plasma glucose (PPG) and glycated hemoglobin (HbA1c).

The concentration of HbA1c predicts diabetes complications because it reflects more harmful glycation sequelae of diabetes, such as retinopathy and nephropathy, which are understood to be due to harmful advanced glycation end products (Weykamp C. et al, 2009 ; Pasupathi P. et al, 2010 and Ken S , 2009). Epidemiological and large randomized clinical trial studies such as Diabetes Control and Complication Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) indicated that HbA1c >7.0 % is associated with a significantly increased risk of both microvascular and macrovascular complications, regardless of underlying treatment (UK Prospective Diabetes Study (UKPDS), 1998; Diabetes Control and Complications Trial (DCCT), 1995). Since it reflects the mean glycemic values in the previous 2–3 months, HbA1c is an indicator for overall glucose exposure integrating both fasting and postprandial hyperglycemia even though their relative contribution is undefined (Monnier L. et al, 2003 ; Rosediani M. et al, 2006).
In the absence of HbA1c test and high level of diabetic problems, post-prandial and fasting plasma glucose estimation have come into practice particularly in developing countries to assess glycemic control (Swetha NK, 2014). A number of studies have shown acceptable correlation between HbA1c levels and FPG and PPG level (Weerarathne TP. et al, 2006).

Unfortunately majority (more than two thirds) of patients in therapeutic aims targeting for HbA1c and FPG has failed to achieve their glycemic goals (American Diabetes Association, 2014 ; Sharon H. et al, 2004). In order to detect diabetics, fasting blood glucose (FBS) is suggested as the best and the most common test with the cutoff point >126 mg/dl (Reinauer H, 2002). The Diabetes Control and Complications Trial suggested the value of 6% as HbA1c cut point (Anonymous, 2008).

On the other hand, HbA1c has some important limitations and is a rather complex measure of hyperglycemia. A large number of medical condition such as the presence of hemoglobin variants, malignancies, hemolytic anemia, and variety of systemic conditions as well as various medications and pregnancy are associated with alterations in the HbA1c values and may provide unreliable information (Kilpatrick ES. et al, 2007; Bloomgarden ZT, 2009).

But still measurement of HbA1c level remains the gold standard for assessment of glycemic control at follow up (Ghazanfari Z. et al, 2010).

2.5.2. KIDNEY PROFILE

Urea and creatinine are good indicators of a normal functioning kidney and increase in the serum are indications of kidney dysfunction (Kamal A , 2014). Creatinine (2-Amino-1-methyl-5H-imidazol-4-one) is a heterocyclic compound with an empirical formula of C₄H₇N₃O. In vivo, creatine is irreversibly and nonenzymatically transformed to creatinine with a steady rate of
approximately 2.0 % per day (Wyss and Kaddurah-Daouk, 2000). In normal subjects, creatinine is excreted primarily by the kidneys. Creatinine is chiefly filtered out of the blood by the kidneys, though a small amount is actively secreted by the kidneys into the urine. The measurement of creatinine levels in human blood or urine is clinically essential because the levels partially reflect the state of renal and muscle function. The typical human reference ranges of creatinine are 0.5-1.0 mg/dl (about 45-90 μmol/L) for women and 0.7-1.5 mg/dl (60-110 μmol/L) for men. Long-term complications from high blood sugar can include kidney failure which may require dialysis, and poor circulation of limbs leading to amputations. Low serum creatinine levels were associated with a higher risk of T2DM in a recent study of non-obese middle aged Japanese men (Harita et al., 2009), leading to speculate that low creatinine might reflect low muscle mass volume. The serum creatinine concentrations indicating renal insufficiency in the Korean patients with type 2 diabetes were considerably lower than those in Caucasians, and the serum creatinine concentration alone exhibited a limited diagnostic value (Lee et al., 2009).

Measurement of serum urea and creatinine are easily available tests for this purpose which can assist in detection and prevention diabetic kidney disease at an early stage and can limit the progression to end stage renal disease.

2.5.3. LIPID PROFILE

Lipids defined as biological substances that are generally hydrophobic in nature and in many cases soluble in organic solvents (Smith A. 2000). Lipids are first absorbed from the small intestine and emulsified by bile salts which are synthesized from cholesterol in the liver, stored in the gallbladder and secreted following the ingestion of fat. The principles of lipid metabolism
in diabetes are acceleration of lipid catabolism, with increased formation of ketone bodies and decreased synthesis of fatty acid and triglycerides.

Triglycerides is the most common type of lipid formed in animals. It contains three fatty acid molecules attached to one molecule of glycerol by ester bond and containing saturated fatty acid which do not have kinks in their structure, pack together more closely and tend to be solid at room temperature. Recent studies have demonstrated that in diabetic patients TG levels is a risk factor for CVD and despite glycemic control, the incidence of macrovascular disease is increased two to five-fold in diabetics as compared to nondiabetic patients. This is attributed mainly to diabetic dyslipidemia (Stamler et al., 1993).

Cholesterol is an unsaturated steroid alcohol containing 4 rings (A, B, C and D). It has single C-H side chain tail similar to fatty acid in the physical properties. In diabetes mellitus the plasma cholesterol level is usually elevated and this plays a role in the accelerated development of the atherosclerotic vascular disease that is a major long term complication of diabetes in human.

Dyslipidemia is common in diabetes and may contribute significantly to the excess risk of cardiovascular disease (CVD) among patients with type2 diabetes (Garg and Grundy., 1990).

2.6. GENETIC FACTORS

2.6.1. RAGE GENE

Receptor for advanced glycation end product (RAGE) is a multiligand member of the immunoglobulin superfamily (Neeper et al., 1992) of cell surface molecules. RAGE is composed of three immunoglobulin domains, one V-type and two C-type domains, with a single transmembrane region and a short high charged cytosolic tail of 43 amino acids necessary for signalling. Its gene is located on chromosome 6p21.3 at the major histocompatibility complex.
locus in the class III region (Bierhaus et al., 2005; Basta, 2008). It comprises 11 exons (spanning 3.27 kilobases) (Sugaya et al., 1994) as well as a 3’ UTR (Lu WX. Et al., 2010).

**Fig 5: RAGE Gene**

To-date the majority of studies support RAGE as a central role in the biology and pathogenesis of AGEs. RAGE was initially identified by its ability to bind and internalize AGEs, however, subsequent studies now suggest that RAGE is a signalling receptor, as ligand engagement modulates cellular function to show RAGE is not likely to be a scavenger of AGEs (Schmidt A. et al., 2001).

### 2.6.2. ROLE OF AGE

Interaction between Advanced glycation end products (AGEs) and receptor for AGEs (RAGE) are supposed to play an important role in the onset and progression of diabetic mellitus (Bucciarelli et al., 2002; Valko et al., 2007) and diabetic microvascular complications (Kiritoshi
et al., 2003; Yamagishi et al., 2011). The interaction of AGE-RAGE can activate several intracellular cascades including NADPH-oxidase/NF-Kb (Huttunen et al., 1999; Wautier et al., 2001) and perhaps p21ras/MAP-kinase/AP-1 pathways (Lander et al., 1997), and then induces oxidative stress, cellular dysfunction, increased vascular permeability, adhesion molecule expression, cytokine production and initiation of coagulation (Schmidt et al., 1994).

The role of AGE in vascular disease was first identified by their ability to cross-link proteins of the vascular wall leading to the thickening of vessels and leakage from the vasculature (Bierhaus A. et al., 1998). AGE has been shown to produce a variety of toxic effects by a number of mechanisms. Firstly, the formation of AGE occurs on the extracellular matrix leading to the trapping of proteins and eventual narrowing of the lumen. Secondly, AGE formation occurs intracellularly through rapid intermediates of glucose metabolism, altering protein structure and function. Thirdly, AGE interact with AGE binding receptors which remove and degrade AGES and activate proinflammatory and prothrombotic pathways.

The formation of AGES occurs from the reactive nature of reducing sugars (i.e. glucose) to undergo nonenzymatic rearrangements with amino groups of proteins and possibly DNA to form irreversible cross-links. Although this mechanism has only been accepted as a plausible pathway implicated in the pathogenesis of vascular disease in the last decade, the underlying biochemical reaction process has been known for almost a century. The in vivo formation of non-enzymatic glycated compounds was first detected in 1969 from studies on chromotagenic mobilities of fast moving, minor hemoglobins from diabetic patients, in particular HbA1C, now routinely used as a clinical tool in the management of glycaemic control in diabetic patients (Rahbar, S. et al., 1969). From this hyperglycaemic-induced colour change, Cerami et al. (Rahbar, S. et al., 1979) postulated the relevance of ‘nonenzymatic glycosylation’ in the sequelae of diabetes, further
evidenced by Podger et al. (Rahbar, S. et al., 1985) who observed that cataracts with this characteristic colour change occur at an average of 10–15 years earlier in diabetic subjects.

The glycation process, otherwise known as the Maillard reaction, is divided into three key stages: the early reactions resulting in the formation of a Schiff base and Amadori products, the rearrangements of these chemical groups and the final reactions forming the classical Maillard Browning products or now known as AGEs (Singh, R. et al., 2001). AGEs were originally shown to form over a period of weeks to months on long-lived cellular proteins, however, evidence suggests that glucose is not the only precursor of AGE, as other aldoses react more rapidly with proteins than glucose, including metabolites from the glycolysis and the polyol pathway (Hamada Y. et al., 1996). This could, therefore, suggest a possible role for aldose reductase and this pathway in the formation of AGE.

AGE/RAGE interaction induces endothelin-1 expression, a potent vasoconstrictor (Quehenberger, P. et al., 1995) and consequently inhibits the production of prostacyclin and nitric oxide, a potentially contributory factor to hypertension in diabetes (Veyssier Belot C. et al., 1999). The inhibition of prostacyclin production has consequences in the initiation of retinopathy. The reduced prostacyclin production by the endothelium result in the loss of pericytes (Yamagishi, S. et al., 1995), the earliest visible marker of diabetic retinopathy (Shepro, D. et al., 1993).

Various metabolic theories have been proposed to explain the relationship which includes increased flux through the aldose reductase pathway, the sustained activation of protein kinase C (PKC) by increased levels of diacylglycerol (DAG), and the non-enzymatic glycation of macromolecules. The most compelling of these theories is the formation of advanced glycation end products (AGEs), evidenced by the findings of the United Kingdom Prospective Diabetes
Study (UKPDS) which indicate for every 1% increase in glycated haemoglobin levels, a 37% increase in microvascular disease was seen (Stratton, I. M. et al., 2001).

2.6.3. ROLE OF RAGE GENE POLYMORPHISM

Several polymorphisms of the RAGE gene had been reported, and their associations with type 2 diabetes mellitus (T2DM) and diabetic microvascular complications including diabetic retinopathy (DR) and diabetic neuropathy (DN) had been investigated.

In vivo and in vitro studies have proven that RAGE contributes to the pathogenesis of diabetic microvascular complications (JiXiong et al., 2003; dos Santos et al., 2005). RAGE is regarded as one of the candidate genes involved in the development of DR, since it is expressed by critical tissues such as endothelium, smooth muscle, mesangial cells and monocytes (Hudson et al., 2001).

2.6.4. 2245 G/A and G82S POLYMORPHISM OF RAGE GENE

A role for genetic susceptibility in the development of diabetic vascular disease is supported by family studies of clustering of retinopathy and neuropathy. The RAGE gene was assessed for the presence of novel polymorphisms within exons and gene regulatory regions which might affect function and expression of RAGE (Hudson B. I. et al.,1999 ; Hudson B. I. et al.,2001 ; Kankova K.et al.,2001 and Poirier O. et al., 2001). At first the 11 exons and 3´ UTR of RAGE were identified and screened and a number of amino acid changes were identified. It included a common variant in intron 8 (2245G/A) region and another in exon 3 (Gly82Ser).

2.6.4.1. 2245 G/A POLYMORPHISM

Most of the RAGE polymorphisms that have been identified comprise either rare coding changes
or are located in non-coding regions (Hudson BI. Et al.,1998 and Kankova K. et al.,2001). sRAGE is produced by alternative splicing of RAGE mRNA, which involves regions between intron 7 and 9 (Schlueter C. et al.,2003). The intron 8 region in which the 2245G/A polymorphism is present could hypothetically be involved in this regulatory process. This polymorphism is of interest due to its relatively high prevalence, and the nucleotide change can be rapidly screened using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. This polymorphism creates a PstI restriction site (CTGCA↑G). The nucleotide change can be rapidly screened by polymerase chain reaction (PCR)-RFLP method. Only a few studies have investigated the relationship between this polymorphism and diabetic complications and these were carried out in limited populations. In this study, we aimed to investigate the association of 2245G/A RAGE gene polymorphisms with neuropathy and retinopathy in the Eastern-Indian type 2 diabetic population.

2.6.4.2. G82S POLYMORPHISM

Gly82Ser polymorphism was found within the ligand-binding domain of RAGE, making this a potentially important gene variation. It is at codon 82 (GGC → AGC) in exon 3 of RAGE and leads to change from glycine to serine within the putative ligand-binding domain of the protein (Hudson BI et al.,1998). The Gly82 and Ser82 isoforms were expressed in cells and the consequences of the Gly82Ser on receptor function were investigated. A number of studies have been performed to assess the prevalence of the Gly82Ser polymorphism in vascular disease of both diabetics and non-diabetics. This polymorphism is particularly interesting because it results in the creation of an AluI restriction site (AG↑CT). The nucleotide change can be rapidly screened by polymerase chain reaction (PCR)-RFLP method. The functional data seen in vitro
with the Gly82Ser polymorphism, supports a role for the Ser82 allele in heightening the inflammatory responses in diabetic neuropathy and retinopathy.

In conclusion, it is highly likely that glycation and the ultimate formation of AGEs are central to the pathogenesis of diabetic disease. From *in vitro* and *in vivo* studies, the interaction with their receptor, RAGE presents a novel target for drug intervention to reduce and prevent the development of the debilitating side effects of hyperglycaemia. Taken together with genetic susceptibility data from RAGE allelic variants which may influence the disease progression further, it may be possible to tailor individual therapeutics against RAGE to ameliorate disease progression.

### 2.7. ENVIRONMENTAL FACTOR

Heavy metals have been known to possess many adverse health effects; still, heavy metal pollution continues, and is even increasing in some parts of the world, in particular in less developed countries (Järup L, 2003). Uncontrolled industrialization has released heavy metal pollution in the world. Heavy metal pollutants damage organ functions and disrupt physiological homeostasis. Diabetes mellitus is growing in prevalence worldwide. Several studies have indicated that the deficiency and efficiency of some essential trace metals may play a role in the islet function and development of diabetes mellitus. Some toxic metals have also been shown to be elevated in biological samples of diabetes mellitus patients. Those toxic metals, when analyzed in human samples, it has been found that the mean concentrations of these heavy metals were significantly higher in scalp hair samples of diabetic patients as compared to control subjects, suggesting that toxic metals may play a role in the development of diabetes mellitus (Afridi HI et al., 2008).
2.7.1. ARSENIC

Arsenic (As) is a naturally occurring toxic metalloid. It could be found as inorganic and organic forms in the environment. The pathway for iAs metabolism in humans involves the reduction of AsV-species to AsIII-species followed by oxidative methylation of AsIII-species, yielding mono and dimethylated metabolites that contain either AsIII or AsV (Thomas DJ . et al.,2007). Arsenic (+3 oxidation state) methyltransferase (AS3MT) is the key enzyme in this pathway(Thomas DJ . et al.,2007 and Lin S. et al.,2002). The trivalent iAs, arsenite (iAsIII), and its methylated metabolites containing trivalent As, methylarsonite (MAsIII) and dimethylarsinite (DMAsIII), are generally more toxic and reactive than their pentavalent counterparts, arsenate (iAsV), methylarsonate (MAsV) and dimethylarsinate (DMAsV) (Styblo M . et al.,2002). These trivalent arsenicals are also potent inhibitors of insulin-stimulated glucose uptake by cultured murine adipocytes (Walton FS.et al.,2004 and Paul DS. Et al.,2007).

2.7.2. ARSENIC AFFECTED AREAS

Uncontrolled industrialization has resulted in a very wide segment of the human population being exposed to agents that have the potential to cause or exacerbate diseases. In Taiwan, the areas along the south-western coast were known to have arsenic contamination in drinking wells or underground water and the hyper-endemic occurrence of a peripheral vascular disease (blackfoot disease) in these area’s villages (Chiou JM. et al.,2005; Tseng WP.,1989; Chen KP. et al.,1962; Lai MS. et al., 1994 and Tseng CH. et al.,2002). In these areas, arsenic concentrations in drinking water were measured and ranged from 0.35 to 1.14 mg/L, with a median of 0.78 mg/L in the early 1960s(Chen KP. et al.,1962). Many studies have also indicated that it was a dose-response relationship between accumulative arsenic exposure and prevalence of diabetes mellitus in the villages of the South-Western coast of Taiwan exposed to arsenic from drinking
water (0.1–15 and >15 mg/L-year). The incidence of diabetes in these areas (the village exposed to arsenic) was two to five times higher as compared with those in the other non-endemic areas (Lai MS. et al., 1994 and Tseng CH. et al.,2002). Moreover, similar findings have also been reported in Bangladesh and others (Afridi HI et al.,2008). Recent study has reported that after adjustment for biomarkers of seafood intake, total urine arsenic (median urine level, 7.1 μg/L) is associated with increased prevalence of type 2 diabetes. The authors suggested that low levels of exposure to inorganic arsenic in drinking water may play a role in diabetes prevalence (Navas-Acien A et al.,2008). From these findings, chronic exposure to arsenic is an important risk factor for induction of diabetes mellitus in an arsenic-contaminated environment. Particularly high arsenic levels have been reported in West Bengal, an Indian province bordering Bangladesh (Datta DV. et al., 1974 ; Garai R. et al.,1984; Guha Mazumder DN. et al.,1992; Guha Mazumder DN. et al.,1988 and Mandal BK. et al.,1996). West Bengal and Bangladesh form a geologic continuity, and the occurrence of arsenic in drinking water seems to depend on arsenic-rich sediments.

2.7.3. GROUNDWATER ARSENIC CONTAMINATION

Arsenic could be easily solubilized in ground water. Natural arsenic in ground water at concentrations above the drinking water standard of 10 mg/liter was not uncommon. Man-made sources of arsenic, such as mineral extraction and processing wastes, poultry and swine feed additives, pesticides and highly soluble arsenic trioxide stockpiles were also not uncommon and had caused the contamination of soil and ground water. Many epidemiological studies have demonstrated that chronic exposure to arsenic in drinking water was associated with the increase in rates of various chronic diseases, including cancers, nervous system diseases, peripheral vascular disease (blackfoot disease (BFD), a peripheral artery disease) and endocrine dysfunction in the United States and other countries (Lewis DR et al.,1999 and Rodriguez VM et al.,2003).
Therefore, the United States Environmental Protection Agency (U.S. EPA) recommended a reduction in the maximum contaminant level (MCL) from 50 μg/L to 10 μg/L for arsenic in public drinking water supplies. Exposure to arsenic through drinking water is a serious problem reported to occur in various countries, including Argentina, Chile, Taiwan, and the United States (World Health Organization. Environmental health criteria, 1981).

Arsenic contamination of ground water and its impact on human health have already been reported from several Asian countries (Mukherjee et al. 2006; Smedley and Kinniburgh 2002). The magnitude is severe in Bangladesh followed by West Bengal of India. An estimated 36 million people in the Bengal Delta, India are at risk from drinking arsenic contaminated water.

Arsenic contamination of ground water in Chandigarh and some areas of Punjab in India were reported in 1976 (Datta 1976). The first As-contamination report in the lower Ganga plain of West Bengal was published in 1984 (Garai et al. 1984). In 1992, As in ground water in the Padma-Meghna-Brahmaputra plain of Bangladesh was first identified (Dhar et al. 1997) and further reported in the International Arsenic Conference in Calcutta in February 1995 (International Arsenic Conference 1995; Chakraborti et al. 2002). In 2001, groundwater As-contamination in the Terai region of Nepal was also reported (Shrestha et al. 2003). In June 2002, As-contamination in the middle Ganga plain of Bihar was detected (Chakraborti et al. 2003). During 2003–2004, As-contamination in Uttar Pradesh, Jharkhand and Assam states in India was revealed (Chakraborti et al. 2004). In 2006, As was detected in the ground water of Manipur, one of the 7 North Eastern Hill states of India (Chakraborti et al. 2008a). Based on the survey over the last 20 years in the Ganga-Meghna-Brahmaputra (GMB) plain (an area of 5,69,749 km2 with a population of over 500 million), it is expected that the ground water of some parts in all states (Uttar Pradesh, Bihar, Jharkhand, West Bengal, Arunachal Pradesh and Assam) in the Ganga-Brahmaputra plain of India and six out of seven North Eastern Hill states
(except the Mizoram) in India will be As-affected. Even it was anticipated from last 20 years arsenic experience in GMB plain and North Eastern Hill states that the flood plains of the country Bhutan on the foothill of Himalaya would be arsenic-affected. Padma-Meghna-Brahmaputra plain in Bangladesh is considered worst affected in world’s as scenario (Smith et al. 2000). Arsenic survey in the villages of West Bengal by School of Environmental Studies (SOES) started in early 1988. At that time, 22 affected villages in 12 blocks/Police stations (PSs) of 5 districts were known. The present results indicated that 3417 affected villages in 111 blocks of 9 highly affected districts are arsenic-contaminated. Although arsenic groundwater contamination in Bangladesh was identified in 1992 (Dhar et al. 1997), detailed survey on As contamination in Bangladesh was initiated in 1996. At that time there was information on 3 affected villages in 2 Police Stations of 2 districts (Dhar et al. 1997). Current findings showed that 2000 affected villages in 189 Police Stations of 50 affected districts are arsenic-contaminated. There is an increasing number of As affected villages with every new survey. After surveying for so many years in the villages of West Bengal, we feel that we have seen only the tip of the iceberg of this calamity.

2.7.4. CLINICAL MANIFESTATION

Arsenic might be impairing glucose metabolism; (Liebl B. et al.,1995) however, only few studies have evaluated that the impairment of insulin secretion in β-cells associated with environmental arsenic exposure in mammals (Diaz-Villasenor A. ET AL.,2006). On the other hand, many studies have indicated that arsenic could alter signaling transduction factors, including NFκB, p38 mitogen-activated protein kinase (MAPK), tumor necrosis factor-α (TNFα), phosphatidylylinositol-3-kinase (PI3K) and PI3K-dependent phosphorylation of protein kinase B (PKB/Akt), and affecting the insulin-stimulated glucose uptake (ISGU) in adipocytes or skeletal muscle cells, which may potentially link with insulin resistance (Le Roith D. et al.,2001
and Somwar R. et al., 2002). PI3K signaling is a pivotal role in the metabolic actions of insulin and its activation regulates multiple signaling transductions. Increased PI3K-mediated PKB/Akt phosphorylation has been reported in β-cells exposed to high dose of arsenic (Souza K. et al., 2001). The phosphorylation of PKB/Akt signaling was also one of the key steps in the activation of glucose transporter 4 (GLUT4) by insulin (Souza K. et al., 2001). Thus, it has been suggested that the exposure to high dose of arsenic might mimic the action of insulin by phosphorylation of PKB/Akt-mediated GLUT4 expression in vitro.

2.7.5. DIABETIC COMPLICATIONS AND ARSENIC

**Diabetic Neuropathy:** Arsenic exposure is associated with wide range of neurological complications in humans such as impaired memory, Parkinson's disease, Guillain-Barré like neuropathy, encephalopathy and peripheral neuropathy (Piao F. et al., 2005 and Felix K. et al., 2005). The mechanism postulated for arsenic-induced neurotoxicity majorly involve oxidative stress with increased reactive oxygen species, lipid peroxides along with decrease in superoxide dismutase, and reduced glutathione levels (Dwivedi N. et al., 2011). Acute arsenic toxicity decreases acetyl cholinesterase activity and hence causes cholinergic crisis like situation with altered mental status and weakness, which can be associated with peripheral neuropathy, neuropsychiatric abnormalities, and extrapyramidal disorders (Patlolla AK. et al., 2005). Moreover, arsenic affects the peripheral nervous system by disrupting the neuroskeletal integrity and thus markedly diminishes the nerve conduction velocity in the peripheral nerves to cause peripheral neuropathy (Bardullas U. et al., 2009).

**Diabetic Retinopathy:** Production of thousands of chemicals has contributed to industrial & economic development in many parts of the world. This trends is however been associated with the release of new chemicals & possibly toxic substances into the environment & food chain
Review of Literature

adversely effecting human health. The results of these toxic by-products are retinal dysfunction, leakage of blood vessels and blindness. Biomarker studies were done regarding health risk linked to environmental pollutant. In diabetic retinopathy where newly formed blood capillaries are already fragile, the conditions become worse due to this toxicity.

2.8. ROLE OF ENZYMES

2.8.1. INTRODUCTION

Aldose reductase is the rate limiting enzyme of the polyol pathway. The nicotinamide adenine dinucleotide phosphate [NAD(P)H]-requiring aldose reductase, catalyses the reduction of glucose to sorbitol followed by the oxidation of sorbitol to fructose by NAD+ dependent sorbitol dehydrogenase. At normal blood glucose concentration (5.5 mM), aldose reductase catalyzed reaction represents less than 3% of total glucose utilization (Morrison, A.D. et al., 1970). However, hyperglycemia results in saturation of hexokinase and more than 30% of glucose is directed into the polyol pathway. In a diabetic state, polyol pathway increases in tissues that do not require insulin for cellular glucose uptake, such as retina, kidney, peripheral nerves and blood vessels (Stephen S.M. et al., 2003). The overall reaction of the polyol pathway leads to a shortage of intracellular NAD(P)H and a surplus of NADH, i.e., a reductive imbalance. Increased NADH generation during conversion of sorbitol to fructose provides substrate for NADH oxidase to generate ROS (Morre, D.M. et al., 2000).

Reactive oxygen species (ROS) is the free radicals which are highly unstable molecules produce by normal cellular metabolism. ROS include superoxide anion (O2·­), hydroxyl (.OH), hydrogen peroxide (H2O2). Free radicals produced under physiological conditions are maintained at steady state levels by endogenous or exogenous antioxidants which act as free radical scavengers. However, oxidative stress occurs when the production of free radicals overwhelms...
the detoxification capacity of cellular antioxidant system causing biological damage (Abdollahi, M. et al., 2004; Ridnour, L.A. et al., 2004 and Halliwell, B. et al., 2011). The endogenous antioxidants comprise of the enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) (Halliwell, B. et al., 2007).

The polyol pathway also results in reduction in the bioavailability of NAD(P)H. The reduced bioavailability of NAD(P)H negatively affects the antioxidant defence system by depleting glutathione (GSH) a very important antioxidant. This is because the activity of GSH reductase, an antioxidant enzyme that generates GSH from its oxidized form (GSSH) depends on NAD(P)H.

Fig 6: Mechanism of polyol pathway induced development of diabetic complications
2.8.1.1. ENZYME ALDOSE REDUCTASE

2.8.1.1.1. BIOCHEMISTRY

Aldose reductase is a small monomeric protein composed of 315 amino acid residues. The primary structure, first determined on rat lens aldose reductase (Carper et al., 1987, 1989), demonstrated high similarities to another NADPH-dependent oxidoreductase, human liver aldehyde reductase (EC 1.1.1.2) (Wermuth et al., 1987). The degree of similarity clearly suggests that these proteins belong to the same family, namely aldoketo reductase superfamily, with related structures and evolutionary origins.

Crystallographic structures have been determined for human aldose reductases (Borhani et al., 1992; Wilson et al., 1992). The enzyme molecule contains a (β/α)₈ barrel structural motif with a large hydrophobic active site. The cofactor NADPH binds in an extended conformation to the bottom of the active site, located at the center of the barrel.

2.8.1.1.2. PHYSIOLOGY

Aldose reductase is a cytosolic enzyme present in most of the mammalian cells, although the distribution of the enzyme is not uniform among tissues. Under normoglycemia, most of the cellular glucose is phosphorylated into glucose 6-phosphate by hexokinase. A minor part of nonphosphorylated glucose enters the so-called polyol pathway, the alternate route of glucose metabolism. The rate-limiting step of this polyol pathway is the reduction of glucose to sorbitol catalyzed by aldose reductase (EC 1.1.1.21). Sorbitol is subsequently converted to fructose by sorbitol dehydrogenase, thus constituting the polyol (sorbitol) pathway.
Fig 7. Polyol (sorbitol) pathway; glucose-6-P, glucose 6-phosphate.

Under hyperglycemia, because of the saturation of hexokinase with ambient glucose, the increased flux of glucose through the polyol pathway accounts for as much as one-third of the total glucose turnover (González et al., 1984). This leads to overflow of the products of the polyol pathway along with depletion in reduced nicotinamide adenine dinucleotide phosphate (NADPH) and the oxidized form of nicotinamide adenine dinucleotide (NAD$^+$), the cofactors used in the pathway. The acceleration of the polyol pathway thus elicits various metabolic imbalances in those tissues that undergo insulin dependent uptake of glucose. Such metabolic perturbation provokes the early tissue damage in the “target” organs of diabetic complications, such as ocular lens, retina, peripheral nerve, and renal glomerulus (Kinoshita and Nishimura, 1988; Pugliese et al., 1991).

2.8.1.1.3. ROLE IN DISEASE

In Diabetic Neuropathy patients, excess glucose not metabolized by glycolysis enters the polyol pathway. There is increased flux through the polyol pathway, resulting in elevated nerve levels of glucose, fructose, and sorbitol, possibly due to enhanced aldose reductase and reduced sorbitol dehydrogenase (Oates PJ et al, 2002). Sorbitol accumulation leads to cellular and osmotic stress.
Aldose reductase uses reduced nicotinamide adenine di nucleotide phosphate (NADPH) to reduce glucose to sorbitol, which is oxidized to fructose by sorbitol dehydrogenase using nicotinamide adenine dinucleotide (NAD$^+$). NADPH is needed to regenerate the antioxidant glutathione, thus promoting oxidative stress. Decrease in cellular NADPH caused by the fluxed in the polyol pathway is referred to as pseudohypoxia and decreases generation of nitric oxide in endothelial cells and alters redox balance (Tesfamariam B., 1994). This complex interaction causes alteration in the redox state, which leads to oxidative stress and increased neuron damage (Nishikawa T et al., 2000). The increased NADH/ NAD$^+$ ratio can alter enzyme activities which contribute to diabetic microvascular complications (Williamson JR et al., 1993).

In Diabetic Retinopathy cases, under hyperglycemic conditions, glucose that enters cells is partly metabolized to sorbitol via the rate-limiting enzyme aldose reductase (the polyol pathway). Sorbitol is then metabolized to fructose, a process that is relatively slow. Sorbitol accumulation within the crystalline lens increases with chronic hyperglycemia (Frank RN, 1994; Peterson CA et al., 1992). It leads to rise in intracellular osmolarity, which causes absorption of water into the cells and cellular swelling. The resultant damage to lens epithelial cells, which have a high concentration of aldose reductase, is responsible for diabetic cataract formation. Aldose reductase is also found in high concentration in retinal pericytes. In this tissue the polyol pathway can result in alteration of cellular metabolism, leading to basement membrane thickening and other diabetic complications (Peterson CA et al., 1992).

**2.8.2. ROLE OF ANTIOXIDANT ENZYMES**

The term “antioxidant” is frequently used in the biomedical literature. These antioxidants are any
substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate” (Halliwell B.et al.,1989). Antioxidants can act at different levels in an oxidative sequence. Antioxidants could act by:

i) Decreasing localized $O_2$ concentrations (e.g., by combining with $O_2$ or displacing it).

ii) Preventing initiation of peroxidation by scavenging species capable of abstracting hydrogen atoms, such as hydroxyl radical, 'OH.

iii) Quenching or scavenging singlet $O_2$ which can react directly with membrane lipids to produce peroxides. For example, lycopene has been reported to be the best lipid soluble quencher of singlet $O_2$ in human plasma (Di Mascio P. et al.,1989).

iv) Binding metal ions in forms that will not generate reactive species (such as 'OH, ferryl, or $Fe^{2+}/Fe^{3+}/O_2$ complexes) and/or will not decompose lipid peroxides to peroxyl and alkoxyl radicals.

v) Removing peroxides by converting them into nonradical products, such as alcohols. For example, glutathione peroxidases act as peroxide-removing antioxidants (reviewed in (Ursini, F. et al.,1987))

vi) Chain breaking, i.e., reacting with chain-propagating radicals (peroxyl and possibly alkoxyl), so preventing continued hydrogen abstraction from fatty acid side chains.

It must be remembered that oxidative damage to DNA or to proteins (e.g., proteins involved in maintaining concentrations of intracellular “free” $Ca^{2+}$ at a very low level, or proteins essential to cytoskeletal structure) may be equally or more important than damage to lipids as a mechanism for mediating oxidative stress (Starke P. et al.,1986: Mirabelli F. et al.,1988 and McConkey D. J.)
et al., 1989). Thus antioxidants have evolved to protect not only lipids, but also proteins and DNA.

Free radicals, which are atoms or molecules with an unpaired electron, are capable of reversibly or irreversibly damaging compounds of all biochemical classes, including nucleic acids, proteins and free amino acids, lipids and lipoproteins, carbohydrates and connective tissue macromolecules (Hemnani and Parihar, 1998). Free radical reactions have been implicated in the pathology of many human diseases including atherosclerosis, ischaemic heart disease, ageing process, inflammation, diabetes, immunodepression, neurodegenerative condition and other disease conditions (Maxwell, 1995).

It is now well-established that the major primary intracellular antioxidant defenses are the enzymes superoxide dismutase, catalase, and glutathione peroxidase (Fridovich I., 1989; Sies H., 1985 and Beyer W. F. Jr., 1988).

2.8.2.1. ENZYME SUPER OXIDE DISMUTASE (SOD)

Superoxide dismutase (SOD) (EC 1.15.1.1) is a family of metalloenzymes which is known to accelerate spontaneous dismutation of the superoxide radical to hydrogen peroxide and molecular oxygen (McCord. J. M. et al., 1969). SOD is widely distributed among aerobically living organisms and has been inferred to play an important role in controlling superoxide levels in cellular compartments (McCord. J. M. et al., 1971 and Fridovich. I. 1978).

2.8.2.1.1. BIOCHEMISTRY

A blue copper protein was isolated from bovine erythrocytes by Mann and Keilin (Mann T. et al., 1939) in 1939. This crystalline protein, called “hemocuprein,” contained 0.34% copper and had a molecular weight of about 34,000. No enzymatic activity was found for this protein. More
recently a copper protein from human erythrocytes has been characterized (Markowitz H. et al., 1959 and Kimmel J. R. et al., 1959). This protein was called “erythrocuprein” and was similar in copper content and size to hemocuprein. Erythrocuprein, like hemocuprein, lacked an apparent enzymatic function. Furthermore, copper proteins of similar size and copper content have been isolated from tissues other than blood. A copper protein called “cerebrocuprein” was isolated from human brain (PORTER, H. et al., 1957). “Hepatocuprein” has been isolated from bovine (MANN T. et al., 1939) and equine (MOHAMED, M. S. et al., 1953) liver. These proteins, like the erythrocyte proteins, have not been associated with any apparent enzymatic function. MCCord, J. M. et al., 1968, led to the proposal of a previously unsuspected enzymatic activity which catalyzes the dismutation or disproportionation of superoxide free radical anions.

The superoxide radical $O_2^-$ is a highly toxic species which is generated by numerous biological and photochemical reactions. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes which catalyse the breakdown of $O_2^-$ according to the equation below and which appear to play an important protective role in vivo (Fridovich, I., 1975; Halliwell B., 1974; Hewitt J. et al., 1975 and Lumsden J. et al., 1975).

$$\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2$$  (MCCORD J. M. et al., 1969)

Reactions dependent on the presence of $O_2^-$ are inhibited by addition of superoxide dismutase (Fridovich, I., 1975) and this observation has led to the development of a number of assays for measuring the activity of this enzyme. The assays usually consist of a system which generates $O_2^-$, e.g. mixtures of xanthine and xanthine oxidase (McCord, J. M. et al., 1969), NADH and phenazine methosulphate (Nishikimi, M., et al., 1972).
2.8.2.1.2. PHYSIOLOGY

In mammals there are three SOD isoenzymes, the cytosolic dimeric CuZn-SOD (SOD1) (McCord and Fridovich, 1969), the mitochondrial matrix Mn-SOD (SOD2) (Weisiger and Fridovich, 1973) and the secretory tetrameric extracellular SOD (SOD3) (EC-SOD) (Marklund, 1982). The first is a dimer (consists of two units), whereas the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, whereas SOD2, the mitochondrial enzyme, has manganese in its reactive centre. The genes are located on chromosomes 21, 6, and 4, respectively (21q22.1, 6q25.3 and 4p15.3-p15.1).

2.8.2.1.3. ROLE IN DISEASE

Oxidative stress has been implicated in the etiology of long-term diabetic complications, including diabetic neuropathy (Hunt V. et al., 1991). Oxygen free radicals (OFR) may damage neurons by causing nerve lipid peroxidation, the breakdown of mitochondrial DNA and inhibition of the respiratory chain, and the cross-linking of the neurofilament protein (Low PA. et al., 1997; Zhu M. et al., 1994; Cameron NE. et al., 1997). Treatment with antioxidants (e.g. probucol, alpha-lipoic acid) decreases lipid peroxidation and oxidative stress in neural tissues and improves the condition of rats with diabetic neuropathy (Nagamatsu M. et al., 1995; Van Dam PS. Et al., 1999). Antioxidant enzyme activity is low in peripheral nerves and even lower in diabetic nerves, possibly due non-enzymatic glycation and autooxidation of the glycated protein (Martinez-Blasco A. et al., 1998; Yan H. et al., 1997). Antioxidant enzymes may protect against the rapid onset and progression of diabetic neuropathy (DN) by reducing the excess of both OFR and peroxide. Defects and mutations in the genes encoding these enzymes may therefore lead to susceptibility to DN.
Retinopathy is a debilitating vascular complication of diabetes. Superoxide radicals are elevated in the retinal mitochondria and their scavenging enzyme, manganese superoxide dismutase (MnSOD), is compromised (Kowluru RA. et al., 1997; Du Y. et al., 2003 and Kanwar M. et al., 2007). This decrease in MnSOD activity is observed as early as 2 months after induction of hyperglycemia in rats (Kowluru RA. et al., 1997), and the enzyme remains compromised at duration when capillary cell apoptosis or pathology characteristic of diabetic retinopathy are observed in the retinal vasculature (Mizutani M. et al., 1996; Kern TS. Et al., 2000; Kowluru RA. et al., 2004 and Kowluru RA. et al., 2007). Prevention of MnSOD inhibition by the administration of antioxidants or overexpression of SOD2 prevents the development of diabetic retinopathy in rodents (Kowluru RA. et al., 2004; Kowluru RA. et al., 2006 and Kowluru RA. et al., 2006), suggesting it has a major role in the development of diabetic retinopathy.

Good glycemic control, if started in the initial stage of diabetes, prevents the development of retinopathy, but if reinstituted after a period of poor control, fails to halt its development, suggesting a metabolic memory phenomenon. Patients in the conventional treatment regimen during the Diabetes Complications and Control Trial had a higher incidence of complications several years after switching to intensive therapy than the patients in intensive control (Diabetes Control and Complications Trial, 2003).

2.8.2.2. ENZYME GLUTATHIONE PEROXIDASE (GPx)

Glutathione peroxidase (GPx) (EC 1.11.1.9) is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. GPx contains selenium and was discovered in 1957 by Gordon C. Mills (Mills GC., 1957). GPx functions in the scavenging and inactivating of hydrogen and lipid peroxides, thereby protecting the body against oxidative stress.
2.8.2.2.1. BIOCHEMISTRY

The main reaction that glutathione peroxidase catalyzes is:

\[ 2 \text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GS}–\text{SG} + 2\text{H}_2\text{O} \]

Where GSH represents reduced monomeric glutathione, and GS–SG represents glutathione disulfide. The mechanism involves oxidation of the selenol of a selenocysteine residue by hydrogen peroxide. Glutathione peroxidase is induced by H\(_2\)O\(_2\) and organic peroxides. This enzyme contains selenium and catalyzes the reaction of glutathione disulfide production. Glutathione reductase is the enzyme co-operating with peroxidise and does not directly participate in the protection of a cell against ROS; however, by reproducing the reduced form of glutathione, its effect increases the protective potential and prevents the development of protein damage. The enzyme, which is directly related with GSH, is glutathione transferase catalyzing the conjunction of glutathione with various electrophilic compounds. The products of its activity are glutathione S-conjugates (Szelachowska M et al.,2002 and Twardowska-Saucha K et al.,1994).

2.8.2.2.2. PHYSIOLOGY

There have been eight different members of the GPx family that have been identified in humans: GPx1, GPx2, GPx3, GPx4, GPx5, Gpx6, GPx7, and finally GPx8. GPx1 is the most abundant of all eight and it is found throughout all the tissues in the human body, whose preferred substrate is hydrogen peroxide, whereas GPx2 is mainly found in the intestines. Glutathione peroxidase 4 (GPx4) has a high preference for lipid hydroperoxides; it is expressed in nearly every mammalian cell, though at much lower levels.
2.8.2.2.3. ROLE IN DISEASE

Oxidative stress may be defined as an imbalance between production and degradation of ROS. One of the major hypotheses to explain the onset of diabetic complications is a DM-induced increase in oxidative stress (Kochar NI et al., 2009). Glutathione peroxidase (GPx), an enzyme whose main biological role is to protect the organism from oxidative damage by free radicals. The red cell has been a central focus of research on GPx because it is thought to undergo a high endogenous rate of H$_2$O$_2$ production from hemoglobin autoxidation (Johnson RM et al., 2000). GPx activity is considered to represent the initial protective response required for adjusting the H$_2$O$_2$ concentration under normal physiological conditions as well as after oxidative insult (Lee YS et al., 2008).