SUMMARY

Diabetes mellitus is a metabolic disorder, which makes it difficult for the cells of the body to convert glucose into energy. It results from reduced ability, expression and action of insulin to stimulate glucose transport. If uncontrolled diabetes stands for long it results in chronic hyperglycemia, which is closely associated with long-term complications. These complications include damage, dysfunctioning and failure of various organs including eyes, kidneys, nerves, heart and blood vessels. The majority of cases of diabetes fall into two broad etiopathogenic categories i.e. type 1 and type 2 diabetes. In Type 1 diabetes, hyperglycemia results from inadequate secretion of insulin by islets of Langerhans in the pancreas. The more prevalent type 2 diabetes results from combination of insulin insensitivity and inadequate compensatory insulin secretary response. In type 2 diabetes, the degree of hyperglycemia is sufficient enough to cause pathological and functional changes in various target tissues.

Diabetes is rapidly becoming the epidemic of 21st century. It accounts for 3.8 million deaths per year globally. According to the latest figures from International Diabetes Federation Diabetes Atlas, a staggering 246 million people across the world are affected by diabetes. Although diabetes is prevalent throughout the world, the prevalence rate of diabetes in India is significantly higher in terms of the number of people affected. Infact, India leads the global top ten countries in the world in terms of highest number of people with diabetes with a current figure of 40.9 million. For this reason India is now referred to as diabetic capital of the world. The staggering growth rate of diabetic patients and its far-reaching social and economic consequences is a public health issue of clinical importance. A number of factors and their complex interplay predispose the individual towards the risk of diabetes. Genetics, among these factors plays a cardinal role in development of disease pathology. An extensive search has been carried out to identify and decipher clues in our genetic makeup and develop “genetic landscape of diabetes” that may explain why some people are at a higher risk for diabetes then others. Family history, which captures these genomic interactions, is considered clinically useful to identify people at high risk. A family history of
diabetes is independently and significantly associated with the development of diabetes itself, even after adjusting for other risk factors. In general, a family history of diabetes in a first-degree relative doubles a person’s risk of developing diabetes. This is attested by a number of studies carried out in this field. Studies have reported that heredity is an important risk factor that contributes toward the development of diabetes. Recent studies have shown the presence of oxidative stress in first degree relatives of type 1 diabetic patients. This shows the contributory effect of oxidative stress towards the development of diabetes. Oxidative stress represents an important pathway for the destruction of cells and production of Reactive Oxygen Species that can result in cellular injury through cell membrane lipid destruction and cleavage of DNA. This can lead to various derangements in the body including diabetes. In contrast to this, only a limited number of studies have been done to identify relationship between family history and development of type 2 diabetes. Hence, the present thesis is focused on identifying and gaining better understanding of relationship, if any, between family history and type 2 diabetes. Our work entails study of various markers of oxidative stress in first-degree relatives of type 2 diabetes to understand relationship between oxidative stress, family history and susceptibility towards type 2 diabetes. The study was conducted in normotensive subjects with age ranging between 30-45 with ≥ one parent diagnosed with type 2 diabetes. They were compared with age and sex matched controls having negative family history of type 2 diabetes. Type 2 Diabetic patients were also included in the study. The study was carried out on human blood samples. The present work entails the detailed study of the following biochemical parameter in the test subjects:

1. **Total Plasma Antioxidant capacity in terms of FRAP values**

   This method measures the reducing power of most important antioxidants such as vitamin C, vitamin E, uric acid and bilirubin. FRAP was analyzed by
reduction of ferric ions to ferrous ions. The method is simple, fast, convenient and reproduces good results. By this we can find out how the antioxidant capacity changes with the progression towards the disease.

The FRAP values were determined following the method of Benzie IFF & Strain JJ, 1996.

2. Erythrocytes Reduced Glutathione (GSH) content

GSH is one of the most important cellular anti-oxidant and cell protectant. It is synthesized from glutamate, cysteine and glycine. GSH plays an important role in anti-oxidant defense, nutrient metabolism and regulation of cellular events including gene expressions, DNA and protein synthesis, apoptosis, signal transduction, cytokine production, immune response and protein glutathionylation. Glutathione deficiency contributes towards oxidative stress, which plays a key role in ageing, and pathogenesis of different diseases.

Erythrocyte GSH content was measured by the method of Beutler E et al, 1963.

3. L-Cysteine influx in erythrocytes

The amino acid L-Cysteine has been termed as the “semi-essential” amino acid. It is the only amino acid that has a free thiol (-SH) functional group. This amino acid is required by erythrocytes for synthesizing glutathione hormone. The redox sensitive cysteine residues of many redox sensitive signaling molecules interact not only with ROS but also respond to change in intracellular thiol/disulphide redox state. Altered thiol status may lead to pathological consequences.

L-Cysteine influx in erythrocytes was measured by the method of Sedlak J & Lindsay RH, 1963.

4. Erythrocyte Malondialdehyde (MDA) content

It is the primary product of lipid peroxidation. It causes tissue membrane
damage by reaction of oxygen with polyunsaturated fatty acids. The erythrocyte membrane is prone to lipid peroxidation under oxidative stress conditions that involves cleavage of polyunsaturated fatty acids at their double bonds leading to the formation of MDA. Increased levels of lipid peroxidation products are found in diabetes, atherosclerosis, liver disease apoplexy and inflammation.

Erythrocyte MDA was measured by the method of Esterbauer H & Cheeseman KH, 1990.

5. **Superoxide Dismutase (SOD) activity**

SOD represents one of the major reactive oxygen species (ROS) dependant enzymes. SOD converts superoxide anion radicals produced in the body to hydrogen peroxide. This enzyme works in a tightly balanced system, and any disruption of this system promotes oxidation. This enzyme is also considered as primary antioxidant enzyme since it is involved in the direct elimination of reactive oxygen species. Increased interest in SOD in recent years is triggered by the role it plays ageing and pathologies including diabetes.

Plasma SOD activity was determined by a modification of the method described by Markland S & Markland G, 1974.

6. **Catalase (CAT) activity**

Catalase is one of the important intracellular reactive oxygen species scavenging enzymes. Catalase catalyzes the reduction of hydrogen peroxide into water and oxygen, and thus protects mammalian cells against oxidative damage. According to literature, the activity of Catalase, like SODs, is modulated by a number of stimuli and is indeed regulated to compensate for the biological requirements imposed by increased oxidative stress.

Plasma Catalase activity in plasma was determined by the modification of Beers RF & Sizer IW, 1952.
7. **Nitric Oxide (NO)**

NO is a vital biological molecule. It is the product of five electron oxidation of amino acid L-Arginine mediated by one of three isoforms of nitric oxide synthase. It plays a significant role in diverse biological processes such as host defense, homeostatic and development function, cardiovascular regulation, signal transduction, neurotransmission and wound healing. Any pathology of NO production in the body can result in numerous disorders and diseases. Plasma NO level was measured as nitrite by Griess reagent as described by **Green L et al, 1982.**

8. **Plasma membrane redox status (PMRS)**

PMRS is the membrane transport system that includes the reduction of extracellular oxidant by using the reducing power of intracellular antioxidants. Ascorbic acid is used as an electron donor, making the cell respond to changes in redox equilibrium. Upregulation of PMRS activity leads to cell survival and membrane homeostasis under stress condition. Thus PMRS may be regarded as inherent compensatory mechanism operating in the human body, which provides protection against increased oxidative stress during various pathological conditions. The activity of erythrocyte PMRS was estimated by method of **Avron M & Shavit N, 1963.**

9. **AFR reductase**

Erythrocytes play a crucial role in recycling ascorbate in blood plasma. The erythrocyte AFR reductase is involved in the reduction of ascorbate free radical (AFR) to ascorbic acid (ASC) in the plasma. Recycling of ascorbic acid from its oxidized forms plays an important role in maintaining the tissue level of this vitamin. This can be explained as secondary compensatory response in the human antioxidant defense system. The erythrocyte AFR reductase activity was measured assayed following the method of **May J et al, 2004.**
10. Sodium Hydrogen (Na/H) antiport

Na/H antiport is one of the most studied plasma membrane transport system that plays an important role in transport of protons across plasma membrane. The sodium/hydrogen exchanger (NHE) plays a key house keeping role in all cells by controlling cell volume, maintaining intracellular pH and regulating response to stimulus and cell proliferation. Abnormal functioning of NHE is linked to pathology of several diseases.

Erythrocyte Sodium/hydrogen antiport activity was measured by the method of Matteucci E et al, 2001.

Overview of Results

- A decrease in plasma total antioxidant status was observed in both type 2 diabetic patients and their first degree relatives. This was in contrast to the control subjects. This is attributed to the compensatory mechanism of antioxidants for suppressing free radical production.

- The level of non enzymatic antioxidant glutathione was decreased in both type 2 diabetic patients and their first degree relatives. This was in contrast to the control subjects. This decrease in glutathione content can be attributed to the decline in antioxidant defense and over production of free radicals in type 2 diabetic patients and their first degree relatives.

- Erythrocyte L-Cysteine influx was decreased in both type 2 diabetic patients and their first degree relatives. Such low levels of L-Cysteine may be responsible for low glutathione content because it is one of the limiting amino acid for glutathione synthesis. Furthermore, this decreased level of L-cysteine explains the presence of cellular stress in type 2 diabetic patients and their first degree relatives.

- Marker of oxidative stress, MDA was abnormal in both type 2 diabetic patients and their first degree relatives in contrast to normal subjects. This suggests the presence of oxidative imbalance in the patients. Furthermore, it also supports the view that familial elements precede diabetes.
• Activity of antioxidant enzyme Superoxide Dismutase and Catalase were also increased in type 2 diabetic patients and their first degree relatives in contrast to normal subjects. This suggests increased production of free radicals in these patients.

• In contrast to normal subjects, increased NO production was observed in type 2 diabetic patients and their first degree relatives. This suggests higher susceptibility of type 2 diabetic patients and their first degree relatives towards cardiovascular risk. It may also serve as marker indicating altered and impaired oxido/reductive status in the test groups, which can potentiate the development of diabetes.

• PMRS as evidenced by rate of extracellular ferricyanide reduction, is selectively increased in both type 2 diabetes and in their first degree relatives when compared to normal subjects. Increased PMRS appears to attenuate oxidative stress acting as compensatory mechanism and provides the cells with survival mechanism by lowering oxidative stress.

• This was also followed by our finding of increased AFR Reductase activity in type 2 diabetic patients showing increased production of ascorbate free radical and hence increased ascorbate recycling.

• The activity of erythrocyte Na/H exchange (NHE, secondary counter transport regulating cell pH, volume and proliferation) is increased in both type 2 diabetic patients and in their non-diabetic first degree relatives. NHE up-regulation, secondary to chronic oxidative stress, further points to familiar redox disequilibrium. Moreover, we believe that increased NHE activity may be an early event in response to any abnormal change in pH, resulting in cellular alkalinization for the regulation of intracellular pH.

Cumulative results from these studies confirm the altered redox balance and oxidative stress in type 2 diabetic patients and their first degree relatives when compared with their respective control. Furthermore, redox imbalance and oxidative stress may be responsible for disturbance of homeostasis in type 2 diabetic families even before the development of the disease. Moreover, based
on the results obtained from our studies of biochemical parameters described above, we may use these biochemical parameters as markers for identifying the group/population with the risk of type 2 diabetes. In addition, identification of risk population with elevated oxidative stress early on may allow the earlier targeting for the specific therapy regarding increase antioxidant intake with an aim to reduce risk factors for future adverse future adverse conditions and increasing oxidative tolerance. Our study may open new avenues for more targeted research in this field.