CHAPTER II
REVIEW OF LITERATURE

Review of Literature is important in any scholarly works or findings since it provides the back drop for choosing dimensions that must be explored for a particular enquiry. The purpose of review of literature is not to list the number of published works but to pick up important findings that relate to the substantive concern of the proposed research (Potti, 2000). This section reviews the important literature related to the study. Previous research studies are abstracted and significant writing of authorities in the area under study is reviewed as it provides background for the development of the present study. The Review of Literature pertaining to the study entitled “Antioxidant Status of Subjects with Diabetes Mellitus” is presented under the following heads:

2.1 The History, Definition And Classification Of Diabetes Mellitus
2.2 Present Scenario
2.3 Etiology Of Diabetes Mellitus, The Predisposing Factors
2.4 Symptoms And Pathophysiology, The Emerging Facts
2.5 Diagnosis Of Diabetes Mellitus, The Critical Control Point
2.6 Complications Of Diabetes Mellitus, A Major Threat
2.7 Reversion Of Diabetes Through Prevention And Treatment
2.8 Dietary Management In Diabetes, A Science Based Approach
2.9 Antioxidant Status In Diabetes Mellitus
2.10 Endogenous – Enzymatic Antioxidants
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2.12 Spirulina, The Green Gold
2.13 Food Supplementation – Related Studies
2.14 Conclusion
2.1. THE HISTORY, DEFINITION AND CLASSIFICATION OF DIABETES MELLITUS

Diabetes Mellitus is a disorder that was recognized in antiquity, but its history has been characterized by numerous cycles of discovery, neglect and rediscovery. It is known to have affected man for many thousands of years, the earliest record is found in the Eber’s Papyrus of Egypt which dates back to 1500 BC. However it was not until the second century AD that Aretaeus of Cappadocia named the disease ‘Diabetes’ the Greek word meaning ‘to flow through a siphon’. According to him “diabetes is a remarkable disorder consisting of a moist and cold wasting of the flesh and limbs into urine. The disease is chronic in its character, and is slowly engendered, though the patient does not survive long when it is completely established, as the marasmus produced rapid and death speedy”. This was a masterly description of the striking symptoms of severe diabetes, a copious flow of urine accompanied by the wasting away of both muscle and fat (Dressler and Johnson, 1982; Dwivedi and Dwivedi, 2007).

In India the disease was recognized from Vedic Age (6th century). The Vedas are the first appearance of the literature in the world. The physicians among Hindu monks recognized that the urine from diabetic patients tasted sweet, although it was not until the 18th century that the sweet tasting substance was identified as the sugar-glucose and the word mellitus or honeyed was added (Geevarghese, 1976). Between 5th and 6th century AD, two notable Indian physicians Susrutha and Charaka first reported the association of Polyuria with sweet tasting substance in urine and named the condition as “madhumeha” (means urine tasting like honey and attracts ants) which is still remembered among Indians (Dwivedi and Dwivedi, 2007).

One of the first clues to the pathology underlying diabetes came in 1889 from the experimental work of Von Mering and Minkowsrski. They found that removal of pancreas from dogs gave
rise to a syndrome resembling diabetes and showed that pancreas is a gland of internal secretion which produced a substance that regulated glucose metabolism. Laguesse in 1894 drew attention to the original observations of Paul Langerhans who had in 1869 described small heaps of islands of previously unknown cells in the pancreas. Laguesse suggested that these islands of cells should be called as the ‘Islets of Langerhans’. The hypothetical pancreatic secretion was given the name ‘Insulin’ by De Meyer in 1900 (Mount, 1985).

As the disease evolved clinically, 19th century has become experimental period during which extensive histological studies and attempts to extract insulin from pancreas were carried out. Sir Frederick Grant Banting and Charles Herbert Best in 1921 produced the first useful and successful insulin preparation for the treatment of diabetes (Dressler and Johnson, 1982). Though a revolution has come in pathogenesis and preventive intervention of diabetes mellitus the development of long term complications and many other aspects still remain as a mystery, as it is a heterogeneous disorder of multi-factorial origin of which many sub types are present. Hence there is every reason to suppose that diabetes will remain as a threat to public health in this century and beyond.

The modern definition for Diabetes Mellitus is that it is a syndrome with metabolic, vascular and neuropathic components that are inter related characterized by alteration in carbohydrate, fat and protein metabolism; secondary to absent or markedly diminished insulin secretion and/or to ineffective insulin action (Davidson, 2001). Diabetes is the leading cause of blindness in working age group, the second commonest cause of lower limb amputation and leading cause of renal failure (Gadsby, 2002).

Appropriate classification of the syndrome is essential for the treatment and for orderly and standardised epidemiological, genetic and clinical research on diabetes mellitus. In 1980 WHO proposed a classification for diabetes which is accepted widely. The WHO classification underwent minor changes in 1985 and 1990.
A. Clinical Classes

1) Type 1 (Insulin – Dependent Diabetes Mellitus, IDDM)
2) Type 2 (Non- Insulin Dependent Diabetes Mellitus, NIDDM)
   a) Obese
   b) Non obese
3) Malnutrition – Related Diabetes Mellitus (MRDM)
4) Diabetes related to other conditions and syndromes are:
   a) Due to pancreatic disease
   b) Disease of hormonal or endocrine etiology
   c) Due to drugs or chemicals induced conditions
   d) Abnormalities of insulin or its receptors
   e) Certain genetic syndromes
5) Gestational diabetes mellitus (GDM)
6) Impaired Glucose tolerance (IGT)
   a) Obese
   b) Non obese

B. Statistical risk classes

1) Patients with previous abnormality of glucose tolerance
2) Patients with potential abnormality of glucose tolerance
   (Page, 2003)

According to Kuzuya et al., (2002) classification of diabetes and related disorder of glycemia include (1) type 1 (2) type 2 and (3) those due to specific mechanisms and disease and (4) gestational diabetes mellitus.

Classification of Diabetes by American Diabetic Association-2003

1) Type 1 Diabetes Mellitus: β cell destruction usually leading to absolute insulin deficiency. It is classified as- a) Immune-mediated and b) Iodio pathic
2) Type 2 Diabetes Mellitus: It may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance
3) Impaired Glucose Homeostasis
4) Gestational diabetes mellitus (GDM)
5) Other specific types:
   1. Genetic defects of beta cell function
   2. Genetic defects in insulin action
   3. Diseases of the exocrine pancreas
   4. Endocrinopathies (e.g., Cushing's)
   5. Drug or chemical induced (e.g., steroids)
   6. Infection (e.g., rubella, Coxsackie, CMV)
   7. Uncommon forms of immune-related diabetes
   8. Other genetic syndromes

Some patients cannot be clearly classified as having type 1 or type II diabetes. Clinical presentation and disease progression vary considerably in both types of diabetes. Occasionally, patients who otherwise have type 2 diabetes may present with ketoacidosis. Similarly, patients with type 1 diabetes may have a late onset and slow (but relentless) progression of disease despite having features of autoimmune disease. Such difficulties in diagnosis may occur in children, adolescents, and adults. The true diagnosis may become more obvious over time (American Diabetes Association 2011).

2.1.1. Type I Diabetes (Insulin Dependent Diabetes Mellitus-IDDM)

In Type I Diabetes Mellitus (Insulin dependent Diabetes mellitus) patients are depended on exogenous insulin for survival due to β cell damage. There is usually sudden onset and there is an inability of the pancreas to produce adequate amount of insulin. This may be caused by either genetic influences or a variety of viruses or neonatal exposure to albumin in cow’s milk or due to autoimmunity (Srilakshmi, 2007; Bowen and Gill, 1997). Inheritance of type I diabetes is polygenic, if the father has type I diabetes the child has 6% risk of developing the syndrome, a 3% risk if mother has and 30% risk if both the parents have the disease. In studies using identical twins if one twin has type I diabetes there is only 30-40% chance that the other twin will develop type I diabetes (Bowen and Gill, 1997; Song and Franz, 2005).
2.1.2. Type II Diabetes (Non-Insulin Dependent Diabetes Mellitus-NIDDM)

In Type II Diabetes Mellitus (Non Insulin dependent diabetes mellitus) develops slowly mostly in middle aged people and is usually milder and more stable. Insulin may be produced by the pancreas but action is impaired, hence the patient is not completely dependent on exogenous insulin (Bamji et al., 1999; Srilakshmi, 2007). Some patients require insulin injections for control of blood sugar due to the inability to comply with long term management programs and it represents the progressive deterioration of quality of life and expectancy. Type 2 diabetes which has progressed to require injected insulin, and latent autoimmune diabetes of adults (or LADA or "type 1.5" diabetes) have been defined by various sources as type 3 diabetes which also includes gestational diabetes and insulin-resistant type1 diabetes (or "double diabetes") (http://www.diabetes.org/other-types.jsp)

Type II diabetes is divided into two main groups obese and non obese, this distinguish the underlying cause and help to determine the most effective treatment. Resistance to insulin can be maintained by weight reduction but it is more complicated in those who are thin or of normal body weight. The prevalence of type II diabetes increases with age and has a strong genetic link. The risk of a child developing Type II diabetes is about 15% if one parent has the disease, 70-75% if both are affected (Braxton et al., 1993; Lynne, 1999) and 60-100% chance for identical twins if the mother is affected coupled with poor intrauterine nutrition (Chiuand, et al., 1997). In the case of genetic predictors of NIDDM, the concordance rate of NIDDM in monozygotic twins has been found to be around 70-80% compared with10-20% in di-zygotic twins. Early onset may occur in children born to two diabetic parents who receive a double dose of NIDDM gene (Lynne, 1999). Type II diabetes or NIDDM is a public health time-bomb that is set to explode by 2010, with high prevalence in urban area. Urbanisation and associated life
style changes adversely affect the risk factors for diabetes unmasking the high genetic tendency existing in the population (Ramachandran et al., 2002). Life expectancy was reduced by up to 10 years in type 2 diabetes with 75% of people dying of cardiovascular disease (Gadsby, 2002).

2.1.3. Gestational Diabetes Mellitus (GDM)

Gestational diabetes is defined as any degree of glucose intolerance, with onset of first recognition during pregnancy, associated with, increased feto-maternal morbidity as well as long term complications in mother and offspring (Kautzky et al., 2004). The WHO (1985) recommends that women with IGT should be included in this category. But diabetic women who have become pregnant are not included in this class. GDM occurs in about 3% of pregnancies in women.

2.1.4. Malnutrition Related Diabetes Mellitus (MRDM)

In tropical developing countries young diabetes with a history of nutritional deficiency and a constellation of symptoms, signs and metabolic characteristics failed to come under the two clinical sub classes of diabetes IDDM and NIDDM. Some young diabetic require high dose of insulin to control blood glucose but don’t develop ketoacidosis on withholding insulin which indicates that they are relatively resistant to ketoacidosis (Bamji et al., 1999). Hence WHO in 1985 included a great number of such cases in the new class of diabetes; Malnutrition Related Diabetes included tropical diabetes, endocrine pancreatic syndrome and ketosis.

2.1.5. Maturity Onset Diabetes of the Young (MODY)

Maturity–onset diabetes of the young (MODY) is rare form of Juvenile diabetes mellitus defined by early onset, absence of ketosis, non insulin dependent diabetes and autosomal dominant inheritance. Molecular genetic testing is very important as it enables us to make a firm diagnosis of MODY. Each child of a parent with
MODY has a 50% risk of developing diabetes. The progression of diabetes and the treatment change according to the gene affected. Hyperglycemia is mild and begins in early childhood. Usually dietary restriction is required except during pregnancy as microvascular complications are rare during the period. It is recently thought to account for 22.5% of all cases of type 2 Diabetes mellitus (Nobre et al., 2002).

2.1.6. Neonatal Diabetes Mellitus (NDM)

Diabetes with onset before 6 months-of-age (Neonatal diabetes mellitus) occurs in approximately one out of every 100,000–300,000 live births. Clinically, NDM subgroups include transient (TNDM) and permanent (PNDM) neonatal diabetes mellitus. TNDM often develops within the first few weeks of life and remits by a few months of age. TNDM is most frequently caused by abnormalities in the imprinted region of chromosome 6q24, leading to over expression of paternally derived genes. Mutations in KCNJ11 and ABCC8, encoding the two subunits of the adenosine triphosphate-sensitive potassium channel on the β-cell membrane, can cause TNDM, but more often result in PNDM. In 40% of NDM cases, the genetic cause remains unknown. Correctly identifying monogenic NDM has important implications for appropriate treatment, expected disease course and associated conditions, and genetic testing for at-risk family members. Early recognition of monogenic NDM allows for the implementation of appropriate therapy, leading to improved outcomes and potential societal cost savings (Naylor et al., 2011).

2.1.7. Categories Of Increased Risk For Diabetes

In 1997 and 2003, The Expert Committee on Diagnosis and Classification of Diabetes Mellitus recognized an intermediate group of individuals whose glucose levels, although not meeting criteria for diabetes, are nevertheless too high to be considered normal. These
persons were defined as having impaired fasting glucose (IFG) (FPG levels 100–125 mg/dl [5.6–6.9 mmol/l]) or impaired glucose tolerance (IGT) (2-h PG values in the OGTT of 140–199 mg/dl [7.8–11.0 mmol/l]). It should be noted that the World Health Organization (WHO) and a number of other diabetes organizations define the cutoff for IFG at 110 mg/dl (6.1 mmol/L) (The Expert Committee on Diagnosis and Classification of Diabetes Mellitus 1997 and 2003).

Individuals with IFG and/or IGT have been referred to as having prediabetes, indicating the relatively high risk for the future development of diabetes. IFG and IGT should not be viewed as clinical entities in their own right but rather risk factors for diabetes as well as cardiovascular disease (CVD). IFG and IGT are associated with obesity (especially abdominal or visceral obesity), dyslipidemia with high triglycerides and/or low HDL cholesterol, and hypertension (American Diabetes Association: Standards of Medical Care in Diabetes—2011).

2.2. PRESENT SCENARIO

Demographic and epidemiological evidences suggest that in the absence of effective intervention of diabetes will continue to increase its frequency worldwide. Thus prevention of diabetes and its consequences is not only a major challenge for future but essential, if health for all is to be an attainable target.

2.2.1. Global Scenario

Diabetes is an iceberg disease, which is rapidly increasing and the prevalence is a significant cause of concern (Frank et al., 2003). Diabetes Mellitus is one of the main threats to human health in the 21st century (Pradeepa and Mohan, 2002). According to WHO (1997), the global prevalence of Type 2 diabetes, will more than double from 135 million in 1995 to 200 million by 2025.

The incidence of NIDDM is highest in economically developed nations, particularly the US, where approximately 6.5% of the
population (17 million people) have either diagnosed or undiagnosed diabetes (Rube and McDonald, 2001).

Diabetes Mellitus is a growing epidemic in both developed and developing countries. The spectacular increase in the incidence and prevalence of this chronic disease is destined to have enormous impact on mortality, morbidity and health care resources. The global number of people with diabetes mellitus is expected to be at least 220 million in 2010 reaching 324 million by 2025 (Jayakumar and Nisha, 2005). At the global level around 3.2 million deaths, every year are attributed to complications of diabetes, at the rate of six deaths every minute. The top 10 countries based on the number of sufferers are India, China, USA, Indonesia, Japan, Pakistan, Russia, Brazil, Italy and Bangladesh (www.diabetesindia.com)

According to Sridhar et al., (2005) the total number of people worldwide with Type II diabetes in 2000 was more than 176 million. By the year 2030, the number is estimated to rise to 370 million. In 2025, the worldwide prevalence of diabetes among adults is expected to increase by 35 percent and total diabetics by 122 percent. It is also estimated that the countries with the largest number of diabetics in 2030 will be India (80.9 million), followed by China (42 million) and United States (30 million). Type II diabetes affects 15 million people in the United States and approximately 150 million diabetics around the world. Diabetes is said to be the seventh leading cause of death in the United States (Ornish, 2004).

The World Health Organization has projected that the global prevalence of Type II diabetes mellitus is on the increase, the degree of which differ between countries and ethnic groups within the country. Diabetes Mellitus is a major health problem throughout the world and is the third most common disease in the world next to cardiovascular disease and oncological diseases (WHO, 2005).

Green et al., (2003) estimated the current global prevalence of Type II diabetes as 150 million patients. Projections suggest that by the year 2025 the number of diabetic patients in the world will reach approximately 800 million.
Forouhi et al., (2005) state that prevalence of diabetes in England was 4.41 percent in 2001. Among them 92.3 percent of them had Type II diabetes and 7.7 percent had Type I diabetes. Diabetes prevalence was estimated to be higher in women (5.17%) than men (3.61%). People from ethnic minority groups had higher crude prevalence than white Europeans. Prevalence increased sharply with age (0.33, 3.37 and 13.92% respectively in those aged 0-29, 30-59 and 60+ years).

In Japan, the total diabetic population is estimated as seven million wherein 95 percent of diabetics have Type II diabetes. It is considered as an increasingly important problem and life style related disease (Matvienko et al., 2001).

Asian Indians have a high prevalence of insulin resistance syndrome, which may be the cause for their great tendency to develop diabetes mellitus (Abate and Manisha, 2001; Misra and Naval, 2004). According to WHO (1999), prevalence of Type 1 diabetes in Asia is relatively low and the prevalence of diabetes in urban dwellers is assumed to be twice as high as in the rural population in India (Ramachandran et al., 2004).

2.2.2. Indian Scenario

Nearly 25% of Indian city dwellers (the sub-population most at risk) haven’t even heard of diabetes (Mohan et al., 2007). According to the Diabetes Atlas of International Diabetes Federation (IDF, 2009) India has 40.9 million people with diabetes followed by China with 39.8 million diabetics. As for the projections, in 2035 India will top the list with 69.9 million diabetic (The Hindu, 2006). India had largest number of persons with diabetes with 23 million cases in 2000, rising to 57 million by the year 2025 (Hilarg, 2003).

One out of the four individuals will be an Indian diabetic in the world while three out of four will be from the developing countries (RSSDI, 2007; Chandaraju, 2005). Recent studies show that up to 10 percent of India’s urban population and two percent of the rural population above the age of 15 years has diabetes (Elizabeth and
Alberti, (2001) points out that type II diabetes mellitus in urban Indian adults had increased from less than three percent in 1970s to greater than 12 percent by 2000 while in rural population it has increased to seven percent. Indians are more susceptible to diabetes, particularly when they are exposed to affluent life styles. It is postulated that intake of high calorie and highly milled refined foods in association with sedentary life may be responsible for the higher prevalence of diabetes in urban Indians and migrant Indians.

World Health Organization estimates that diabetes in India would increase to 57.2 million in 2025, from 30 million in 2002 and India will become the diabetic capital of the world (Alberti, 2001). Prasad, (2002) cautions that twenty one million Indians are presently suffering from diabetes; India’s contribution to the diabetic global population would be a whopping sixty million by 2010.

The epidemiological survey has revealed the prevalence of Type II diabetes mellitus in semi urban areas to be almost the same as urban areas, but only 2.9 percent in tribal (Podsedek, 2007). India is considered as the diabetic paradise of the world, in view of the high prevalence of diabetes mellitus in the country. The approximate prevalence in an urban area is around 13.5 percent and in rural it is around three percent (Paulose, 2005). Bhattacharjee, (2004), reports that India has the largest number of diabetic patients in the world. Diabetes prevalence in urban India range from 16 to 20 percent and in rural it is about four percent. As high as 63 percent of diabetics in India are not aware of the fact that they have diabetes and hence exposed to diabetes related complications.

The prevalence of diabetes mellitus is showing a rising trend in Kashmir valley, life style changes and aggressive control of the risk factors are urgently needed to tame this trend. The prevalence of diabetes mellitus was 6.05%, with known diabetes mellitus being 4.03% of the study population and undiagnosed diabetes mellitus being 2.02% subjects (Ahmad et al., 2011).

The prevalence of diabetes and impaired glucose tolerance were 12.1 percent and 14 percent respectively in urban areas, with
no gender difference. Diabetes and impaired glucose tolerance showed an increasing trend with age. The national study shows that the prevalence of diabetes is high in urban India. The prevalence of diabetes is higher in men than in women. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people, greater than 65 years of age (Sarah et al., 2004).

Insulin dependent diabetes mellitus (IDDM) or juvenile onset diabetes mellitus or Type 1 diabetes accounts for about 10 percent of diabetics. Non insulin dependent diabetes mellitus (NIDDM) is the most common type accounting for over 90 percent of all cases of diabetes. About 80 percent of people with Type II diabetes are overweight (www.diabeticindia.com, 2006). The urban diabetes prevalence is reported in the range of 9-16% and in rural India; the prevalence is reported to be 2-4%. In Western India, there was a significant prevalence of diabetes and insulin resistance syndrome (Gupta et al., 2003).

Anjana et al., (2011) found that, till date there has been no national study on the prevalence of diabetes which is truly representative of India as a whole. The data on diabetes complications is even scarcer. There is an urgent need for a large well-planned national study, which could provide reliable nationwide data, not only on prevalence of diabetes, but also on pre-diabetes, and the complications of diabetes in India. Prevalence studies of this nature will have enormous public health impact and help policy makers to take action against diabetes in India.

2.2.2.1. South Indian Scenario

India is experiencing an epidemic of Type 2 diabetes mellitus (DM) in young adults. Study conducted by Raghupathy et al., (2007), in Vellore and nearby villages’ reports high prevalence of glucose intolerance even among young adults in south India. The prevalence of Type 2 Diabetes and impaired glucose tolerance (IGT) was higher in urban than in rural subjects highlighting an urgent
need for preventive action to avert a public health catastrophe in India.

The Chennai Urban population study (CUPS) showed that prevalence of diabetic retinopathy, nephropathy and neuropathy is not very difficult in urban south Indians as compared to that reported among Europeans (Pradeepa and Mohan, 2002; Shanthi Rani et al., 2006).

A series of recent population-based medical studies have revealed that Kerala is on the fast track to become an ‘ailment society’. With a sedentary lifestyle and high intake of saturated fat, the society is increasingly getting vulnerable to creeping maladies that are claiming an increasing number of young lives. Unless ‘Malayalis’ took a look at their diet and limbered up a bit, the state could be in for real shocks on the health front.

Managing diabetes has become easy with modern technology. But treatment has become high-tech, sophisticated and moneyed affair, handled only by diabetologists using the latest type of insulin and devices like the insulin pen or pump, all of which are prohibitively expensive. The staff reporter of the online edition of India’s National Newspaper, (The Hindu, Oct 25, 2010) reports that the physicians of Thiruvananthapuram have appealed to the government to evolve a financial aid scheme for the treatment of children with Type 1 diabetes. Children having Type 1 diabetes require a lifetime’s supply of insulin, the expense of which is unaffordable for most parents.

It is estimated that there are about 1.5 million diabetics in Kerala. The figure could be higher because of the absence of any established population-based health screening programmes, there could be a lot of undetectable cases in the community says V. Mohanan Nair, CEO of the Indian Institute of Diabetes. Less than 50 percent of the patients may have the classic symptoms of diabetes, while the rest are mostly non symptomatic (Maya 2005).

The prevalence of diabetes in Kerala is more than 17%. Majority of diabetic patients are above the age of 60 years. Diabetes
has evolved as a major cause of disability in old age. Newly diagnosed patients are mostly between the age group of 35 and 40 years. 91.2% of diabetic patients’ regularly taking medications have unacceptably high blood sugar values. Though doctors prescribe medications for prevention of heart attack, kidney diseases etc., 85% of the patients do not continue it because of fear of side effects due to drugs and the extra expenses involved (Kesavadev, 2006).

A situational analysis of the burden of diabetes and its determinants was conducted by Medical Trust Hospital and Diabetes Care Centre, Kulanada in a rural village in Central Kerala during January to August 2007. The survey was conducted among the adult residents of two Panchayat wards namely Pathanamthitta District Panchayath and Alappuzha District Panchayath. The overall crude prevalence of diabetes mellitus was 14.6 percent. It is evidenced that Diabetes Mellitus is fast percolating into rural community as well. The crude prevalence of diabetes mellitus among men was 16.5 percent and among women was 13.5 percent. Among the 241 diabetics, 198 (12.0 percent) were already known diabetics whereas the rest 43 (2.6 percent) were newly detected. Nearly (156)10% of population had high fasting serum cholesterol above 240mg and another 28% had cholesterol between 200 and 240. Around 35% were either obese or overweight (BMI above 23). Among men 70% and among women 85 % have central obesity (www.knowdiabetes.com)

Among the diabetic prevalence rate in different districts of Kerala, high prevalence rate of 20 percent was found in the Ernakulam district, (Diamond, 2011) and in Kochi 19.5 percent (Menon et al., 2006).

In Southern Kerala the overall prevalence of NIDDM is 5.9%, highest in urban (12-14%) followed by midland (8.1%), high land (5.8%) and coastal regions (2.5%). Prevalence of NIDDM among urban residents in Thiruvananthapuram city is high (Soman et al., 1999). In an Industrial cohort study conducted by Ajay et al., (2008),
in Trivandrum it was found that the prevalence rate is 16.6 percent. Mohan et al., (2008) conducted Urban and Rural study and found the prevalence in Trivandrum was 11.2 percent.

Kutty et al., (2000) found that the overall prevalence of type 2 diabetes in 30-64-years of age at Neyyattinkara Taluk of Thiruvananthapuram district in Kerala was 9.2% among men, 7.4% among women, and 8.2% for all persons. Mortality rates are twofold higher in diabetics compared to the non diabetic subjects. Effective prevention of diabetes mellitus is a long term programme, which includes activities targeted against all ages from foetal life to elderly and needs to be culturally socially and economically appropriate (Jayakumar and Nisha, 2005).

As the incidence of diabetes on the rise among the younger population, Kerala state might have to deal with an entire generation of chronic diabetes in the next 10-15 years (Soman et al., 1999). Though it is largely known that diabetes leads to very serious and expensive complications, patients are seen only treating the complications rather than implementing stringent steps in preventing them. Only very rarely, Keralites are seen investing money and time, in preventive health care which involves periodic medical examination, extensive laboratory evaluation, diet, drugs and daily exercises based on expert advice (Kesavadev, 2006).

2.3. ETIOLOGY OF DIABETES, THE PREDISPOSING FACTORS

The etiology is the predisposing factors that increase the risk of getting a particular disease. The factors like age, gender, heredity, diet, stress, infection, genetic factors, obesity and ethnicity are involved in the etiology of type II diabetes (Banga et al., 2005).

Insulin resistance and impaired βcell function will lead to hyper insulinemia or relative insulin deficiency that ultimately leads to diabetes mellitus. In 1962 Neel put forward the “thrifty gene hypothesis” which proposed that individuals living in harsh
environment with unstable food supply would maximize their probability of survival, if they could minimize the storage of surplus energy. When the energy storing genotypes is exposed to abundant food it leads to glucose intolerance and NIDDM (Moran, 2009).

Thrifty genotype hypothesis suggests that intrauterine malnutrition leads to defective pancreatic development and to metabolic adaptations that become programmed for the rest of the individuals’ life especially if they become obese in later life. Such individuals may become susceptible to diabetes, hypertension and heart disease in later life (Ballard et al., 1993).

2.3.1. Heredity

Age, gender and heredity play an important role in the onset of diabetes and make diabetics more prone to chronic complications (Kakuri et al., 2001; http://en.wikipedia.org/wiki/Diabetes_mellitus_type_1). Type 1 Diabetes is a polygenic disease, meaning many different genes contribute to its expression. Depending on locus or combination of loci, it can be dominant, recessive, or somewhere in between. The strongest gene, IDDM1, is located in the MHC Class II (Major Histo-compatibility Complex) region on chromosome 6, at staining region 6p21. This is believed to be responsible for the histocompatibility disorder characteristic of type 1 insulin-producing pancreas cells (beta cells) to display improper antigens to T Cells. This eventually leads to the production of antibodies that attack these beta cells. Weaker genes are also located on chromosomes 11 and 18 (Donner et al., 2007).

Neelam and Elizabeth, (2005) opine that non-insulin dependent diabetes has a genetic predisposition and the chances of developing diabetes if someone in the family has diabetes is 20 percent, if one parent has diabetes – 40 percent; if one parent had diabetes and the other parent is from a diabetic family – 70 percent; and if both parents are diabetics – 90 percent.
Velumani (2005) states that, the cause of metabolic syndromes that leads to diabetes is both genes and bad environment. Insulin resistance is an important risk factor for Type II diabetes and coronary heart disease. Genetic factors, intra-uterine environment, early childhood and adult environment are the factors relevant in determining adult insulin resistance (Ebrahim et al., 2004). Genetic characteristics and faulty life style behavior were the reasons for increased diabetic patients in diabetes family (Nicolette et al., 2004).

Yajnik, (2003) opines that susceptibility to Type II diabetes could be due to genetic factors intra-uterine programming, accelerated childhood growth and life style factors. Hereditary background of diabetes and low birth weight increased the risk of abnormal glucose metabolism such as impaired fasting glucose and impaired glucose tolerance in early middle age (Mogren et al., 2003).

2.3.2. Overweight and Obesity

Obesity is an escalating threat to human health worldwide. There are numerous theories as to the exact cause and mechanism in type 2 diabetes. Central obesity (fat concentrated around the waist in relation to abdominal organs, but not subcutaneous fat) is known to predispose individuals to insulin resistance. Abdominal fat is especially active hormonally, secreting a group of hormones called adipokines that may possibly impair glucose tolerance. Obesity is found in approximately 55% of patients diagnosed with type II diabetes (http://en.wikipedia.org/wiki/Diabetes_mellitus). According to Matvienko et al., (2001) and Mokded et al., (2003) obesity is associated with clinical Problems like cardiovascular disease, high blood pressure and Type II diabetes. According to Page, (2003), both genetic and environmental factors influence the development of obesity.

Obesity means excessive storage of fat in the adipose tissue. Overweight and obesity are risk factors for cardiovascular diseases and metabolic diseases (Knypl, 2002). A study conducted at
children's hospital of Philadelphia (The Hindu, 2004) stated that exposure to videogames and television increases the risk of obesity in children which in turn leads to various other complications like hypertension, cardiovascular diseases and diabetes at a younger age.

In grossly obese adolescents both insulin resistance and impaired insulin secretion contribute to the elevation of glycemia and the degree of obesity is related to cardiovascular risk factor independently of insulin resistance (Invitti et al., 2004).

The concurrent rise in weight and obesity, which accompanies Type II diabetes 80 percent of cases interferes with diabetes treatment and exacerbates the likelihood of hypertension, dyslipidemia, atherosclerosis, and polycystic ovarian syndrome (Hensrud, 2005). Prevalence of obesity (body mass index > 25 Kg/m²) was 36.82 % more among the diabetic subjects; central obesity, and family history were significantly associated with the presence of diabetes mellitus (Ahmad et al., 2011).

Dudeja et al., (2001) opine that the association of obesity with Type II diabetes is complex and is compounded by several heterogeneous factors. BMI is directly associated with glucose intolerance. This suggests that increase in body weight, although within the ideal levels of BMI, confers a high risk to Type II diabetes. Obesity is considered to be a risk factor for Type I diabetes in children, acting as an accelerator for the clinical manifestation (Mainouse and Koopman, 2004).

2.3.3. Insulin Resistance

Insulin resistance plays a pivotal role in the metabolic disorders which include blood pressure, dyslipidemia, glucose tolerance and coronary heart disease. The clustering of all these disorders is collectively termed as Insulin Resistance Syndrome (IRS). Overall, 11.2% of population had evidence of Insulin Resistance Syndrome. Prevalence of IRS among males was 12.9% and females 9.9% (Fujimoto, 2002)
2.3.4. Age

Diabetes is more likely to affect older people, although there are people of all ages with the disease. Almost 27% of people of age 65 years and older had diabetes in 2010. About 215,000 people younger than 20 years have diabetes (type 1 or type 2). This represents 0.26% of all people in this age group (The National Diabetes Fact Sheet, 2011). The prevalence of diabetes increases markedly with age. Maximum incidence of non-insulin dependent type of diabetes occurs above the age of 35 years. High proportion of Indians develops NIDDM at much younger age and therefore the prevalence of maturity onset diabetes of young is higher in India (Bamji et al., 1999; Raghuram, 1999).

2.3.5. Infections, Injuries, Drugs and Toxins

Destruction of βcells occurs due to certain viral infections, injuries, drugs or toxins. Human Islet cells in tissue culture could be infected by Coxsackie B4 and Mumps or German Measles viruses. Replication of the cells caused cell death. Half the population is sometimes affected with this virus but less than 1% develops diabetes. Genetic factors may be another reason for this difference. Accidents or injuries that damage the pancreas also destroy the βcell. The drugs alloxan and streptotocin are known to be toxic to the βcells and are capable of causing cell destruction (http://en.wikipedia.org/wiki/Diabetes_mellitus_type_1).

2.3.6. Sex

In most of the countries in the world women are more prone to diabetes than men, especially married women who have had lots of children, as they tend to be fatter than the women who do not have had any children. Diabetes stands out as an area in which clear differences exist in terms of body composition, hormonal milieu, and the psychological variations between the sexes that
accentuate differences in response to a complex, chronic, metabolic disease (Peters, 2011).

Women who develop diabetes while pregnant (gestational diabetes) have a 35% to 60% chance of developing type 2 diabetes in the next 10 to 20 years (The National Diabetes Fact Sheet, 2011). Suzuki and Kono, (1980) suggest that the bigger the baby the greater are the mothers chances of developing diabetes in later years, more over pregnancy produces physiological and psychological stress to the mind which may be a causative factor. Ahmad et al., (2011) found significant difference between males and females (3.6% vs 8.3%, p <0.05) diabetic subjects. There was also significant increase in the prevalence of diabetes mellitus with increasing age (age 20-40 years: 3.02% vs > 60 years 16.66%, P<0.05).

2.3.7. Life Style Pattern

Kapur, (2002) cautions that, only a structured lifestyle, with diet control and exercise can curb the complications in diabetics. Treatments for diabetes aims at relieving the symptoms of diabetes, preventing long term complications through education, careful dietary management and weight control, medication, physical activity, self testing and foot care (www.eeseehealth.com).

People living in cities and urbanized states have a higher risk of developing diabetes as they are less active, lead more stressful lives and have a more fatty diet (Pillai, 2006). Losing weight and taking more exercise could reduce the danger of developing diabetes by nearly 60 percent in people at risk. Cigarette smoking causes specific effect on people with diabetes and is even more intricate and macro vascular and micro vascular complications ensue more quickly in smokers with diabetes (Justin and Sherman, 2005). Janka and Michaelis, (2003) point out that diabetes risk factor can be reduced by non-smoking campaigns and low cholesterol diets. Rossing et al., (2003) state that cigarette
smoking has been associated with development of persistent microalbuminuria as well as nephropathy in diabetic patients.

**2.3.8. Exercise**

Lack of exercise, a poor diet and smoking were associated with significantly increased risk of diabetes, even after adjustment of the body mass index. The majority of cases of Type II diabetes could be prevented by the adoption of healthier lifestyle practices (Hu et al., 2002). Regular physical activity is an important lifestyle factor associated with a reduced incidence of both cardiovascular disease and Type II diabetes (Anderson et al., 2003).

Currently most promising approach to mitigate Type II diabetes in lifestyle intervention is weight reduction, decreased total and saturated fat consumption, increased physical activity with appropriate pharmacotherapy (Knowler and McAulay, 2005).

Losing weight and taking more exercise could reduce the danger of developing diabetes by nearly 60 percent in people at risk. Lifestyle interventions including moderate to intense physical activity such as walking for two and a half hours every week and exercise for 20 to 30 minutes is effective in preventing the diabetic complications (www.nih.gov/index.html).

According to the Research Society for the Study of Diabetes in India (RSSDI - 2007) release, Type II diabetes is responsible for 90-95 percent of diabetes and 80 percent of Type II diabetes is preventable by changing diet, increasing physical activity and improving the living environment. Increased physical activity is associated with significant reduction of mortality. The exercise training is effective in preventing the depression in myocardial glucose metabolism observed in diabetic rat. This may explain the benefits of exercise in preventing cardiac dysfunction in diabetes (Broderick et al., 2005).
2.3.9. Diet

Improper dietary habits significantly increase the risk for the development of Non-Insulin Dependent Diabetes Mellitus (Szponar and Ewa 2002).

Halkjaer et al., (2004) suggested that, diet and alcohol intake patterns are related to the development of obesity. Caffeine induces high levels of cortisol which may promote abdominal fat accumulation. Increased consumption of eggs and saturated fats is associated with significant increase in mortality (Trichopoulous et al., 2007).

Diet alone or exercise alone or diet and exercise combined have all shown promise in reducing incidence of Type II diabetes mellitus (Jayakumar and Nisha, 2005).

2.3.10. Cigarette Smoking

Cigarette smoking causes specific effect on people with diabetes and is even more intricate and macro vascular and micro vascular complications ensue more quickly in smokers with diabetes (Justin and Sherman, 2005). Janka and Michaelis, (2003) point out that diabetes risk factor can be reduced by non-smoking campaigns and low cholesterol diets. Rossing et al., (2003) state that cigarette smoking has been associated with development of persistent micro-albuminuria as well as nephropathy in diabetic patients.

Tiffany and Joshu, (2002) suggest that smoking can result in devastating health consequences for patients with diabetes. Prevention and treatment of smoking should be of high priority for diabetes care providers. Avoidance of smoking, regulating life style practices, relocation by meditation and yoga and stress free life diminishes the risk of diabetes mellitus (Davies and Williams, Deccan Chronicle, 2005).
2.3.11. Alcohol Consumption

Lando et al., (2005) indicate that alcohol consumption is a lifestyle factor that has been suggested to be relevant with respect to the risk of diabetes mellitus. Alcohol intake increases the risk of hyperglycemia and may induce ketoacidosis, lactic acidosis and may contribute to peripheral neuropathy (Sahay and Rakesh, 2002). Moderate to high alcohol consumption was positively associated with incidence of diabetes (Weiman, 2005).

2.3.12. Stress

Stress has a major role to play in the causation and progression of diabetes, particularly in developing countries (www.diabetescare.com). With increasing affluence and mechanization of civilized societies, people are becoming increasingly sedentary (Sengupta and Maju, 2005). Studies from World Health Organization indicate that up to 80 percent of cardiovascular diseases and up to ninety percent of Type II diabetes mellitus and one third of cancers could be prevented through healthy lifestyle changes (Patel, 2002).

Systemic stress may contribute to insulin resistance (Misra and Naval, 2004) and play a key role in the etiology of diabetic complications (Giammarioli et al., 2004), such as cataract, nephropathy, neuropathy (Osawa and Joji, 2005), atherosclerosis and obesity. Post-pyramidal oxidative stress is attenuated when dietary antioxidants are supplied together with a meal rich in oxidized or oxidisable lipids (Sies et al., 2005).

Depressive symptoms impart subsequent physical symptoms of poor glucose control by influencing patient’s ability to adhere to self-care regimen. More aggressive management of depression among patients with diabetes may improve their physical health as well as mental health (Mc kellar et al., 2004).

The diagnosis of NIDDM and its metabolic conditions were associated with an increased frequency of some symptoms in menopausal women. They had more prevalent emotional
symptoms than non diabetic menopausal women and are associated with years since diagnosis and body mass index. (Malacara, 1997).

2.4. SYMPTOMS AND PATHOPHYSIOLOGY OF DIABETES MELLITUS, THE EMERGING FACTS

2.4.1. Symptoms Of Diabetes Mellitus

The classical symptoms of diabetes are Polydipsia, Polyuria and Polyphagia; Polydipsia or excessive thirst is a method of restoring the water content of the tissues lost by Polyuria. The mechanism responsible for Polyuria or increased volume of urine output is based on the amount of glucose in circulating blood and accumulation of ketone bodies in blood acts as diuretics (Tierney et al., 2002). Figure 2.1., shows initial symptoms of diabetes affecting various parts of the body.

Figure 2.1. The Initial Symptoms Of Diabetes Affecting Various Parts Of The Body

The inability of the tissues to use glucose leads to ravenous appetite as the calorie loss through urine stimulates the patient to consume more or breakdown its own fat and protein for energy needs and to protect the vital proteo-plasmic structure from breakdown and the condition is termed as Polyphagia. Mobilization of fat stores and gluconeogenesis from protein leads to weight loss. Dehydration due to loss of large quantity of electrolytes shows soft eye balls, loss of skin elasticity and dryness of tongue and larynx (Arlan and Janet, 2003).

Symptoms may develop quite rapidly in type 1 diabetes, particularly in children; in type 2 diabetes symptoms usually develop much more slowly and may be subtle or completely absent (Tierney et al., 2002).

When the glucose concentration in the blood is raised beyond its renal threshold, reabsorption of glucose in the proximal renal tubule is incomplete, and part of the glucose remains in the urine (glycosuria). This increases the osmotic pressure of the urine inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst (Arlan and Janet, 2003).

Depression in patients may impart subsequent physical symptoms of poor glucose control by influencing patient’s ability to adhere to self-care regimen. More aggressive management of depression among patients with diabetes may improve their physical health as well as mental health (Mc Kellar et.al, 2004).

Prolonged high blood glucose causes glucose reabsorption that leads to changes in the shape of the lenses of the eyes, resulting in vision changes, rapidly in type 1 and more gradually in type 2 diabetes. Type 1 diabetics may initially present with diabetic ketoacidosis (DKA), an extreme state of metabolic dysregulation characterized by the smell of acetone on the patient’s breath; a
rapid, deep breathing known as Kussmaul breathing (Wikipedia. Ketoacidosis, 2007); polyuria; nausea; vomiting and abdominal pain; and any of many altered states of consciousness or arousal (such as hostility and mania or, equally, confusion and lethargy). In severe DKA, coma may follow, progressing to death. Diabetic ketoacidosis is a medical emergency and requires immediate hospitalization (http://en.wikipedia.org/wiki/Diabetes_mellitus).

2.4.2. Pathophysiology Of Diabetes Mellitus

According to Moran (2009) only a fraction of individuals who are genetically susceptible actually develop diabetes. An unknown environmental factors trigger early in life leads to T cell mediated insulitis in susceptible individuals. Figure 2.2., shows the physiological changes leading to diabetes.

![Figure 2.2. Physiological Changes Leading to Diabetes](source: Moran, (2009))

As beta-cells are injured, diabetes auto-antibodies begin to appear in the circulation which can be used as a marker of early disease. Initially, insulin secretion is intact. As beta-cell mass declines, first the response to intravenous glucose is lost, then oral glucose intolerance develops (Moran, 2009).

At this time diabetes presents with frank hyperglycemia, 10-20 percent of beta-cells remain, although these are destined to be ultimately destroyed. The theory behind oral tolerizing protocols
states that repeated gut exposure to beta-cell antigens results in production of T regulatory lymphocytes, which block the destruction of beta-cells by auto-reactive T lymphocytes. Antigen-presenting cells such as the dendritic cell present antigens at the MHC site (Major Histo-compatibility Complex) to T-cells. A co-stimulatory signal is also required, to activate T-cells. Activated T-cells proliferate and begin the immune response against the antigen (Moran, 2009).

With the development of hyperglycemia, loss of insulin secretory capacity results, this may further cause reduction in β-cell function leading to ‘glucose toxicity’. With increasing hyperglycemia, insulin concentration falls so that there is an inverted, U shaped relationship between fasting glucose and plasma insulin (Green et al., 1993 and Nielson et al., 1996). Figure 2.3., shows the inverted ‘U’ shaped relationship between fasting glucose and plasma insulin level.

![Figure 2.3. Inverted ‘U’ Shaped Relationship Between Fasting Glucose And Plasma Insulin Level](image)

β-cell defects may lead to increased hepatic glucose production, reduced insulin and glucose uptake by muscle and excessive lipolysis in adipocytes. Hyperglycemia may also cause secondary insulin resistance by affecting insulin receptor activity and a number of other defects in the insulin signaling pathway which in turn aggravates hyperglycaemia and high circulating NEFA (Non
Esterified Fatty Acid) again causing impaired β cell function (http://care.diabetesjournals.org).

2.5. DIAGNOSIS OF DIABETES, THE CRITICAL CONTROL POINT

The criteria used to diagnose diabetes and impaired glucose tolerance was recommended by WHO in 1985. These criteria were originally based on longitudinal studies in the US and UK which demonstrated that subjects with 2-hour post-challenge values above 200mg/dl (11.1mol/l) were at risk of developing diabetes related complications (Heine, 1996). For decades, the diagnosis of diabetes was based on plasma glucose criteria, either the fasting plasma glucose (FPG) or the 2-h value in the 75-g oral glucose tolerance test (OGTT) (American Diabetes Association 2010).

In 2009, an International Expert Committee that included representatives of the ADA, the International Diabetes Federation (IDF), and the European Association for the Study of Diabetes (EASD) recommended the use of the A1C test to diagnose diabetes, with a threshold of ≥6.5% (5), and ADA adopted this criterion in 2010 (American Diabetes Association 2010). The diagnostic test should be performed using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay. Point-of-care A1C assays are not sufficiently accurate at this time to use for diagnostic purposes (American Diabetes Association 2011).

2.5.1. Glycosylated Haemoglobin (HbA1C)

Glucose sticks to the haemoglobin to make a 'glycosylated haemoglobin' molecule, called haemoglobin A1C or HbA1C. The more glucose in the blood, the more haemoglobin A1C or HbA1C will be present in the blood. Diabetes may be defined as having an
HbA1c >6.5%; 'pre-diabetes' or 'at risk of diabetes' if between 6.0-6.5% and not diabetic if <6.0% (Cowie et al., 2010).

The A1C has several advantages to the FPG and OGTT, including greater convenience, since fasting is not required, greater pre-analytical stability; and less day-to-day perturbations during periods of stress and illness. These advantages must be balanced by greater cost, the limited availability of A1C testing in certain regions of the developing world, and the incomplete correlation between A1C and average glucose in certain individuals (American Diabetes Association 2011). In addition, A1C levels can vary with patients' ethnicity (Ziemer et al., 2010) as well as with certain anemias and hemoglobinopathies. For patients with an abnormal hemoglobin but normal red cell turnover, such as sickle cell trait, an A1C assay without interference from abnormal hemoglobins should be used (www.ngsp.org/interf.asp). For conditions with abnormal red cell turnover, such as pregnancy, recent blood loss or transfusion, or some anemias, the diagnosis of diabetes must employ glucose criteria exclusively (American Diabetes Association 2011).

**2.5.2. Criteria for Diagnosis by World Health Organization (WHO)**

According to the current definition, two fasting glucose measurements above 126 mg/dL (7.0 mmol/l) is the diagnostic criteria for diabetes mellitus. World health organization has laid down the following values for diagnosis of Diabetes mellitus. Table 2.1., shows the WHO criteria of diagnosis for diabetes mellitus.

<table>
<thead>
<tr>
<th>Blood glucose</th>
<th>Whole Blood Glucose (Mg/dl)</th>
<th>Plasma Glucose (Mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Venous</td>
<td>Capillary</td>
</tr>
<tr>
<td>Fasting</td>
<td>&gt; 120</td>
<td>&gt; 120</td>
</tr>
<tr>
<td>2 hours after glucose load</td>
<td>&gt; 180</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

Source: [http://www.who.int/diabetes/publications/en/].
The state of glycemia may be classified within three categories; diabetic type, border line type and normal type. Diabetic type is defined when fasting plasma glucose (FPG) is 7.0 mm/1 (126 mg/dl) or higher and/or plasma glucose 2 hour after 75 g glucose load (2hPG) is 11.1 mmol/1 (200mg/dl) or higher and are considered to have impaired fasting glucose. Normal type is defined when FPG is below 6.1 mmol/1 (110 mg/dl) and 2hPG below 7.8mmol/l (140 mg/dl). Border line type includes those that are neither diabetic nor normal type (Kuzuya et al., 2002).

Patients with plasma glucose at or above 140 mg/dL or 7.8mmol/L, but not over 200, two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance which is a major risk factor for progression to full-blown diabetes mellitus as well as cardiovascular disease (Santaguida et al., 2008). The plasma glucose level depends on the balance between glucose entering and leaving the extra cellular fluid (ECF). The plasma values are higher than whole blood glucose because RBC’s contain less water (73%) than plasma (93%) (Kuzuya et al., 2002).

### 2.5.3. Oral Glucose Tolerance Test (OGTT)

Gestational diabetes mellitus is diagnosed by an oral glucose tolerance test (OGTT) or fasting glucose concentrations in the diabetic range. If the fasting plasma glucose exceeds 95 mg/dl, 180 mg/dl in 1 hour and 155 mg/dl in 2hour after glucose loading the woman is classified as Gestational Diabetes Mellitus. If blood glucose levels cannot be maintained in the normal range insulin therapy should be initiated (Gavin, 2000).

The patients at high risk for diabetes development are called pre diabetic patients. They have higher base line serum glucose concentration, increased body mass index, elevated systolic blood pressure, and reduced serum high density lipoprotein (Segura et al., 2005). Hill, (2005) suggested that rapid, equivocal diagnosis of diabetes is essential to avoid the
onset of complication of diabetes. The signs and symptoms can vary with individuals and are not deniable for diagnosis.

2.6. COMPLICATIONS OF DIABETES, A MAJOR THREAT

Diabetes represents group of metabolic disorders. Poorly managed diabetes can lead to a host of long term complications like heart attacks, stroke, blindness, kidney failure, blood vessel disease, nerve damage and impotence (Banga et al., 2005). Complications of diabetes can be broadly divided into micro vascular disease such as diabetic retinopathy and diabetic nephropathy and macro vascular disease, such as accelerated atherosclerosis and they are the main cause for morbidity and premature mortality among diabetic patients (Yu and Timothy, 2005).

Diabetes is the seventh leading cause of death and can lead to permanent disability and poor health. People with diabetes can experience numerous serious and deadly complications, including heart disease and stroke, blindness, chronic kidney disease, and amputations. The risk for stroke is 2 to 4 times higher among people with diabetes. Adults with diabetes have heart disease death rates about 2 to 4 times higher than adults without diabetes. Diabetes is the leading cause of new cases of blindness among adults aged 20–74 years. Diabetes is also the leading cause of kidney failure, accounting for 44% of new cases in 2008. More than 60% of leg and foot amputations not related to accidents and injuries were performed on people with diabetes. In 2006, that amounted to 65,700 amputations (The National Diabetes Fact Sheet, 2011).

2.6.1. Cardiovascular Diseases

Among diabetic complications cardio vascular complications such as ischemic heart disease, myocardial infarction, angina pectoris and hyperlipidemia become more probable with the longer duration of diabetes and are more likely to develop at an earlier age. Premature atherosclerosis contributes to 75% of death among
individuals with both type I and type II diabetes. Longer the duration of diabetes higher is the incidence of complications (Mageshwari and Minitha, 2004).

According to Kastorini et al., (2011) cardio vascular disease is the major cause of morbidity and mortality. Diabetes mellitus and hypertension are both major health problems in India which co-exist frequently resulting in significant morbidity and mortality. The leading cause of morbidity and mortality in people with non-insulin dependent diabetes mellitus is cardiovascular disease caused by macro and micro vascular degeneration (Rube and McDonald, 2002). Individuals exhibiting precursor symptoms of diabetes mellitus or reaching diagnostic thresholds for diabetes are at increased risk of death due to cardiovascular diseases (Nesto, 2004).

According to Reaven, (2000) a 30 percent jump in insulin levels increased the odds of heart trouble by 70 percent. A study conducted at Chennai with urban population revealed that the prevalence of peripheral vascular disease was 6.3 percent among diabetic subjects compared to 2.7 percent among non-diabetic subjects (Premalatha et al., 2000). Coronary artery disease mortality and the incidence of non fatal coronary artery disease events were 2 to 4 times higher in Type II diabetics compared to age matched non-diabetic subjects (Micha et al., 2010).

Hu and Manson, (2001) opine that chronic degenerative disorders such as diabetes mellitus, cardiovascular diseases, certain types of cancer and osteoporosis are on the rise. Type II diabetes and cardiovascular diseases are important causes of concern due to their near epidemic incidence. The diabetic related complications include cardiovascular diseases, stroke, nephropathy, retinopathy, neuropathy and amputations.

People with diabetes are twenty five times more likely to develop blindness, seventeen times more likely to undergo amputations, two to four times more likely to develop myocardial infarction and twice more likely to develop a stroke than non-diabetics (Pradeepa and Mohan, 2002). Patients with diabetes are two
to three times more likely to develop cardiovascular disease than the individuals without diabetes but proper diabetes management and metabolic control can reduce this risk (Abraham, 2004).

Hypertension is a major threat to diabetics and is caused due to increased peripheral vascular resistance, hyperinsulinemia and insulin resistance. Manchanda, (2000) states that, people with diabetes mellitus have higher risk for heart attacks. The reason for higher incidence of heart attacks in diabetics are clustering of cardiovascular disease factors like abnormal blood pressure, elevated cholesterol, obesity or overweight that predisposes an individual to development of blocks in the blood vessels of the heart (Prasad, 2002). Impaired glucose metabolism is associated with an increased risk of cardiovascular events and cardiovascular associated mortality (Rodriguez, 2008).

Diabetes doubles the risk of stroke in the diabetics. If high blood pressure is prevailing the risk is even greater. World Health Organization, (2002) cautions that heart diseases accounts for approximately fifty percent of all deaths among people with diabetes in industrialized countries. The burden of cardiovascular diseases and disability among people with diabetes is growing at an alarming rate and India will have 60 percent world's cardiovascular diseases below the age of 40 years by 2010.

Heart disease strikes people with diabetes twice as often as people without diabetes. People with diabetes are five times more likely to have heart disease and stroke and once having had a stroke, are two to four times as likely to have a recurrence. Seventy percent of people with diabetes have high blood pressure. Over the past 30 years, deaths from heart disease in men with diabetes have increased by 13 percent compared to 36 percent decrease in men without diabetes (http://w3.whosea.org/women/chap2-lhtml#diabetes).

Mafauzy, (2005) evaluated the status of diabetes care and prevalence of diabetic complications in Asia. He found that there was a high complication rate with the commonest being neuropathy (30.1%) followed by background retinopathy (23.5%), albuminuria (22.9%) and microalbuminuria (20.4%). He concluded that the
majority of diabetic patients treated at the primary care level were not satisfactorily controlled and this was associated with a high prevalence of complications.

2.6.2. Neuropathy

Diabetic neuropathy is the most common complication of diabetes. This can lead to sensory loss and damage to the limbs and it is the leading cause of lower extremity amputations not related to injury. About 67,000 people undergo diabetes related lower extremity amputations each year (www.healingnutrition.com). Fifty percent of diabetics have some form of neuropathy, and develop nerve problems at any time, but longer a person has diabetes, the greater the risk. The highest rates of neuropathy are among people who have had the disease for at least 25 years. The prevalence of autonomic nervous system dysfunction is not precisely known, however tests of autonomic function have shown impairment in nearly 20 to 40 percent of diabetic patients (Mehta et al., 2002).

World Health Organisation, studies (2002) suggest that up to 50 percent of people with diabetes are affected with neuropathy. To some degree neuropathy can lead to sensory loss and damage to the limbs.

Diabetic neuropathy, a peripheral nerve disease in diabetes is the most prevalent type of neuropathy in Japan and contributes to various disabled status in diabetics (Kikkawa, 2000). The overall prevalence of neuropathy in India appears to lower than Europeans. In a study conducted at Chennai with urban population, the Prevalence of neuropathy was 7.7 percent (Ramu et al., 2000). About 60-70 percent of People with diabetes have mild to severe forms of nervous system damage, severe nerve disease and a major contributing cause of lower extremity amputation (www.niddk.com).

Weiman, (2005) points out that diabetes mellitus affects 5 to 10 percent of the U.S. population and it produces one of the serious chronic complication peripheral neuropathy. The peripheral neuropathy in the lower extremities leads to plantar foot ulceration.
Secondary infection of these ulcers is the leading cause of major amputations of feet and legs.

Diabetic neuropathies can be classified as reversible or chronic. The most common type is the chronic progressive distal symmetric polyneuropathy where sensory symptoms in the lower limbs dominate. Automatic neuropathy is often a feature of progressive polyneuropathy, but is rarely symptomatic. The overall prevalence among diabetic patients is around 20-30%. The prevalence increases with the duration of the disease as well as with poor glycemic control, height and age. These patients often lose their protective sensibility and at high risk of foot ulcers and amputations. (Nokleby and Berg, 2005)

Diabetic patients have 12 times higher risk of amputations when compared with non-diabetic subjects. Also patients with impaired glucose tolerance are associated with dysfunction in peripheral nerves and abnormal nerve function. Increased thickening of the small blood vessel is associated with neuropathy in impaired glucose tolerance and diabetic subjects (The Hindu, 2006).

Cardiovascular autonomic neuropathy (CAN) is the most clinically important and well studied form of diabetic autonomic neuropathy because of its association with a variety of adverse outcomes including cardiovascular deaths (Kaveer et al., 2004).

Neuropathy places the foot at increased risk for developing corns, calluses, blisters and ulcerations. If left untreated serious infections may result (www.actus.com). Diabetic foot ulcers precede 85 percent of non traumatic lower extremity amputations. Approximately, three to four percent individuals with diabetes currently have foot ulcers and develop infections (www.emedicine.com).

In India, among the total diabetic population, 15 to 20 percent have foot problems; loss of limb in these patients is preventable as majority of diabetic foot problems India is neuropathic (Bal, 2002). It is one of the costly complications of diabetes, especially in communities with inadequate foot wear. It results from both vascular and neurological disease process, concludes World Health Organization (2002).
More than 60 percent of non traumatic lower limb amputations occur among diabetes. The risk of a leg amputation is 15 to 40 times greater for a person with diabetes. Amputation rates are 1.4 to 2.7 times higher in men than women with diabetes (http://www.sandylake diabetes.com/prevalence 2006).

2.6.3. Nephropathy

Diabetic nephropathy is a very important cause and contributor for chronic renal failure in India (Agarwal, 2002). Strict dietary measures and lifestyle changes in newly detected Type II diabetic patients could yield very good results in controlling and further progression of micro albuminuria, hyper glycemia a and proteinuria (Singh et al, 2001).

Diabetes control and complications trial has clearly shown that intensive therapy reduces the occurrence of microalbuminuria by 39 percent, proteinuria by 54 percent and clinical nephropathy by 60 percent (Buse, 2001).

Nilka et al., (2005) from their study conclude that between 1990 and 2001, the annual number of new patients starting treatment for diabetes related end stage renal disease in the south west American Indians total population increased from 154 to 320, per 10,000 populations. The increasing incidence of diabetes related renal diseases parallels the growing prevalence of diabetes.

Host Etter and Lising, (2003), opine that diseases largely contributing to the end stage renal disease populations are diabetes, mainly Type II diabetes and hypertension. Further, the micro vascular complications of diabetes can be mitigated by careful glycemic therapy. From 1988 to 1997, the incidence rate of the number of early stage renal disease patients doubled in United States, and the two important factors associated with this dramatic rise are the increasing prevalence of diabetes and high blood Pressure (Cowie et al., 2010).

Each year about 28,000 people with diabetes develop kidney failure, and annual total of nearly 100,000 people with diabetes
receive treatment for this condition, (www.healingnutrition.com). Dietary protein intake, salt restriction and restricted intake of saturated fatty acids may have an important role in the prevalence and treatment of diabetic nephropathy (Holler et al., 2000).

2.6.4. Retinopathy

Retinopathy is a progressive vision disorder that can lead to blindness (http://www.who.int/hpr/gr.fs.diabetes.html, 2005). Nearly all patients who have Type I diabetes for about 20 years are likely to have evidence of diabetic retinopathy. Up to 21 percent of people with Type II diabetes have retinopathy when they are first diagnosed with diabetes, and most would eventually develop some degree of retinopathy glaucoma, cataract and corneal disease that contribute to the high rate of blindness. In diabetes cases occurring before the age of 30, men develop retinopathy more rapidly than women.

Vivian (2003) opines that 3 year screening interval could be safely adopted for patients without retinopathy, but yearly or more frequent screening is needed for patients with higher grades of retinopathy. Marianne et al., (2003) reveals that despite modern diabetes management, 39 percent of young adult diabetic patients develop retinopathy within the first 10 years of the disease.

James et al., (2004) points out that the most severe stage of retinopathy, proliferative retinopathy was evident 15 years after diagnosis in 30 percent with Type I diabetes, 10 to 15 percent with Type II diabetes treated with insulin and 5 percent not treated with insulin.

Gardiner et al., (2007) states that diabetic retinopathy is the most widespread complication of diabetes mellitus and a major cause of blindness in the working Population of developed countries. They also further affirm that many neural and micro vascular abnormalities occur in the retina-of short-term diabetic animal’s but-it remains uncertain how closely these acute changes relate to chronic human disease. Proper vision is hindered as the dietary Glycemic Load increases, Schaumberg, et al., (2004) points out that the risk of age related cataract may also increase with dietary Glycemic Load.
2.6.5. Hyperglycemia

Hyperglycemia causes the auto-oxidation of glucose, glycation of proteins and the activation of poly-metabolism. These changes accelerate generation of reactive oxygen species and increase in oxidative chemical modification of lipids, DNA and proteins in various tissues (Osawa and Joji, 2005). Costly complications of diabetes often arise from poor glycemic control. Appropriate diabetes and self care management may improve glycemic control and it is acquired by greater self care and greater self efficacy (Sousa et al., 2005).

Diabetic ketoacidosis an acute metabolic complication of diabetes is characterised by hyperglycemia, acidosis and ketosis and is often as a result of lack of treatment (Charfen and Madonna, 2005). Diabetic pregnancy and associated hyperglycemia needs proper management as it leads to increased rate of congenital malformations despite extensive clinical efforts to normalize the risk for the offspring. Very high doses of dietary antioxidants may be needed to normalize the development of the offspring in pregnancy but that treatment with such high doses may also have adverse effects in non-diabetic pregnancy (Page, 2003).

2.6.6. Diabetic foot infections

Neuropathy places the foot at increased risk for developing corns, calluses, blisters and ulcerations. If left untreated serious infections may result (www.actus.com). Diabetic foot ulcers precede 85 percent of non traumatic lower extremity amputations. Approximately, three to four percent individuals with diabetes currently have foot ulcers and develop infections (www.emedicine.com).

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2.7. REVERSION OF DIABETES THROUGH PREVENTION AND TREATMENT

2.7.1. Latest Developments For Reversion Of Diabetes

Genetic Research can once reverse the epidemic diabetes through the production and distribution of either the Bio-artificial pancreas or Islet cell regeneration or Stem cells approach or Gene therapy or prevention through Immunization. A biological approach to the artificial pancreas is to implant bio-engineered tissue containing islet cells, which would secrete the amounts of insulin, amylin and glucagon needed in response to sensed glucose and hormones(www.isletmedical.com)(http://www.mayoclinic.com/health/islet-cell-transplant).

Technology for gene therapy is advancing rapidly as there are multiple pathways possible to support endocrine function, with potential to cure diabetes. Gene therapy means, designing a viral vector to deliberately infect cells with DNA to carry on the viral production of insulin in response to the blood sugar level. Hence gene therapy can be used to manufacture insulin directly: as an oral medication, consisting of viral vectors containing the insulin sequence, is digested and delivers its genes to the upper intestines. Those intestinal cells will then behave like any viral infected cell, and will reproduce the insulin protein. The virus can be controlled to infect only the cells which respond to the presence of glucose, such
that insulin is produced only in the presence of high glucose levels. As the insulin producing intestinal cells die off, they are boosted by additional oral medications (www.sciam.com/gene_therapy_for_diabetes).

A new method, autologous nonmyeloablative HSTC, was recently developed by a research team composed of scientists from the US and Brazil. This was originally tested in mice and in 2007 there was the first trial with fifteen patients. Recently this trial was continued and 8 more patients were added. In the trial, the researchers implanted diabetes type1 patients with their own stem cells raised from their own bone marrow. The stem cell transplant led to an appreciable repopulation of functioning insulin-producing beta cells in the pancreas so the patients became insulin free. Most of these patients became insulin independent for a mean period of 18.8 months. At the present time, autologous nonmyeloablative HSCT remains the only treatment capable of reversing type 1 DM in humans (Couri et al., 2009).

Microscopic or nanotechnological approaches are under investigation like- implanted stores of insulin metered out by a rapid response valve sensitive to blood glucose levels and closed-loop insulin pumps (Couri et al., 2009). If a biochemical mechanism can be found that prevents the immune system from attacking beta cells, it may be administered to prevent commencement of diabetes type 1. Several groups are trying to achieve this by causing the activation state of the immune system to inhibit inflammation (www.jci.org).

Sun et al., (2011) demonstrated for the first time that a combination of two family of proteins called Advanced Glycation End Products (AGEs), which are increased by high blood sugar levels is associated with protection against eye disease in type I diabetic subjects. There exists a protective molecular, physiologic or genetic mechanism that can fight against the toxic effects of high blood sugars over many decades. Among the population, 43% are free from advanced diabetic eye complications, 87% from kidney
New findings by UT-Southwestern Medical Center, USA researchers suggest that Type 1 diabetes could be converted to an asymptomatic, non-insulin-dependent disorder by eliminating the actions of a specific hormone. These findings in mice show that insulin becomes completely superfluous and its absence does not cause diabetes or any other abnormality when the actions of glucagon are suppressed. Glucagon, a hormone produced by the pancreas, prevents low blood sugar levels in healthy individuals. It causes high blood sugar in people with type 1 diabetes. These findings suggest that “if there is no glucagon, it doesn't matter if you don't have insulin,” (Lee et al., 2011)

2.7.2. Therapeutic And Lifestyle Intervention— Need Of The Hour

Healthy lifestyle choices, including diet and exercise, are necessary. Lifestyle modifications will help to improve glycemic control and prevent or minimize complications of diabetes. The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, with a “Western-Life style and Diet”. Treatment goals cannot be achieved without life style intervention (Rodriguez, 2008).

Healthy lifestyle choices can help prevent type II diabetes and manage type I diabetes. Even if diabetes runs in the individual's family, diet and exercise can help prevent the disease. Healthy lifestyle choices can help individuals prevent potentially serious complications of diabetes, such as stroke, nerve damage, and heart disease (Wild et al., 2004).

Modern lifestyle has taken its toll on our digestive system. Refined, processed, low fiber foods, animal fats, environmental chemicals, lack of exercise and an ever increasing level of stress all contribute to our current gastrointestinal health crisis (Knowler et al., 2002).
Kapur, (2002) cautions that, only a structured lifestyle, with diet control and exercise can curb the complications in diabetics. Treatments for diabetes aims at relieving the symptoms of diabetes, preventing long term complications through education, careful dietary management and weight control, medication, physical activity, self testing and foot care (www.eeseehealth.com).

Regular physical activity is an important lifestyle factor associated with a reduced incidence of both cardiovascular disease and Type II diabetes (Anderson et al., 2003). Stress has a major role to play in the causation and progression of diabetes, particularly in developing countries (www.diabetescare.com). With increasing affluence and mechanization of civilized societies, people are becoming increasingly sedentary (Sengupta and Maju, 2005). Studies from World Health Organisation indicate that upto 80 percent of cardiovascular diseases and up to ninety percent of Type II diabetes mellitus and one third of cancers could be prevented through healthy life style changes (Patel, 2002).

Diet alone or exercise alone or diet and exercise combined have all shown promise in reducing incidence of Type II diabetes mellitus, (Jayakumar and Nisha, 2005). The exercise training is effective in preventing the depression in myocardial glucose metabolism observed in diabetic rat. This may explain the benefits of exercise in preventing cardiac dysfunction in diabetes (Broderick et al., 2005).

Lifestyle interventions including moderate to intense physical activity such as walking for two and a half hours every week and exercise for 20 to 30 minutes is effective in preventing the diabetic complications. (www.nih.gov/index.html)

According to RSSDI (2007) release, Type II diabetes is responsible for 90-95 percent of diabetes and 80 percent of Type II diabetes is preventable by changing diet, increasing physical activity and improving the living environment. Increased physical activity is associated with significant reduction of mortality. Increased consumption of eggs and saturated fats is associated with significant increase in mortality (Trichopoulous et al., 2007).
Bastica et al., (2006) state that diet counseling helps to know about the quality and quantity of food intake and importance of nutritional intake during diseases. Nutrition counseling provides appropriate feeding, contributing to better health conditions and well being. Gallegos et al., (2006) state that diet counseling and educational model applied in the intervention of diabetes was effective to improve the metabolic control of diabetic patients. Vasanathamani and Rekha, (2006) have established that diabetes can easily be controlled by giving organized counseling on diet.

Lifestyle modification through diet counseling prevents the onset of type II diabetes and 58 percent reduction in the progression of the disease can be achieved due to weight loss (Brekke et al., 2006). Diet counseling helps to know about the quality and quantity of food intake and importance of nutritional intake during diseases. Nutrition counseling provides appropriate feeding, contributing to better health conditions and well being (Bastica et al., 2006).

Ramachandran, (2004) says that the progression of impaired glucose tolerance to diabetes is high in native Asian Indians. Both lifestyle modification and diet counseling significantly reduce the incidence of diabetes in Asian Indian with impaired glucose tolerance. Recommendations of diabetes self management education have become a standard component of most treatment guidelines (Clementi, 2000). Patients with diabetes and their families provide 95 percent of their care themselves hence as a consequence, educational efforts to improve self-management are central components of any effective treatment plan (Glasgow and Anderson, 2000).

In self management of diabetes, diabetic education plays a major role in patients. Diabetes care, particularly among older patients, frequently occurs in conjunction with the management of other health problems such as cardiovascular disease, arthritis or hypertension (Goodnick et al., 2001).

Tenfil and Ritenbaugh, (2004) identified development of a primary prevention program gained insight in the diabetes prevention program. The program strived to enhance knowledge of diabetes and
support increased physical activity, increased fruit and vegetables intake and reduced soft drinks consumption. Results indicated a significant reduction in soft drink consumption suggesting a decline in the incidence of hyper insulinenia.

Valk et al., (2005) states that ulceration of the feet, result in amputation and is one of the major health problems for people with diabetes. Therefore he assessed the effectiveness of patient education counseling on the prevention of foot ulcers in diabetics. He concluded that patient education counseling reduced foot ulceration and amputations. Foot care knowledge and behavior of patients seem positively influenced by patient education in the short term. According to Goldhaber-Fiebert et al., (2004), glycemic control of Type II diabetes patients can be improved through community based health intervention addressing nutrition counseling and exercise.

Gucciardi et al., (2007), studied the effect of individual counseling in conjunction with group education among Portuguese Canadian adults with Type II diabetes. He concluded that competent group education in conjunction with individual counseling is more efficacious in shaping eating behaviors than individual counseling alone. According to Pollock et al., (2004), when patients with diabetic foot ulcers were advised on foot care, they followed better practice of foot care which led to the prevention of foot ulcers and amputations. According to the opinion of Eaton, (2002), diet counseling on high risk patients help to improve their health status through counseling. Vikram et al., (2006), opine that the early identification of simple clinical, anthropometry, and biochemical parameters which are strongly associated with early onset of Type II diabetes among young adults in India, along with providing nutrition counseling helps to improve the health status among the young diabetics.

Franz et al., (2007), opines that the role of diet counselor is to assist persons with the metabolic syndrome to make life style changes and modify factors that increase the risk of diabetes. According to Bonometti (2006), the impact of diet counseling for pre-diabetics can prevent or delay Type II diabetes. The diabetes
prevention programme revealed a 58 percent reduction in the progression of diabetes for individual with glucose intolerance that made them incorporate life style changes resulting in weight loss and increased activity. Aruna, (2002) has rightly quoted that "Genetics loads the gun, lifestyle pulls the trigger".

Kanchan and Rajbir (2006) opine that nutrition counseling improved the dietary intake and anthropometric dimension of Type II diabetes mellitus patients, indicating the importance of counseling in the management of the disease. According to Mansourch et al., (2006) the impact of diet counseling is useful among urban diabetics than the rural diabetics, because lifestyle changes in urban area bring increased prevalence of diabetes among young adults in India. Lisa et al., (2006) suggest that diet counseling improves nutritional status and medical status compared with patients who have not received diet counseling among rural Kentucky people in U.S.A. According to Feldeisen and Tucker, (2007) diet counseling interaction is likely to contribute interesting information that may lead to further individualized dietary guidance in the future for diabetic patients.

Simkin-Silverman et al., (2011) opines that patient counseling via the Internet may enable the dissemination of high-quality lifestyle advice to prevent diabetes in patients whose schedules or geographic constraints prohibit their participation in in-person counseling sessions.

2.8. DIETARY MANAGEMENT IN DIABETES, A SCIENCE BASED APPROACH

A low calorie diet extends median and maximum lifespan in many animals. This effect may involve a reduction in oxidative stress. Frequent consumption of vegetables, the soft fleshy edible plant product that contributes vitamins and minerals to the human diet (Nalwade et al., 2003) throughout the year was inversely associated with the risk of blood pressure (Alonso et al., 2004) and NIDDM. This association was maintained after adjustment for age,
gender and family history (Williams et al., 1999). After the diet consisting of daily intake of 700-1000g of fruits and vegetables, dietary antioxidants, redox status markers, parameters of metabolic control were increased in plasma and erythrocytes. There was a rise in reduced glutathione accompanied by a reduction in body mass index and cholesterol (Giammarioli et al., 2004).

Spirulina, a micro-alga which is gaining popularity as a food supplement reduces the blood sugar level. There was significant reduction in body weight and body mass index among males and females after supplementation with spirullina (Anuradha and Vidhya, 2001).

Incorporation of fibre in the diet improve the glucose tolerance in diabetes, i.e. foods high in fibre helps in improving glycemic control, lowering serum cholesterol and triglyceride values (Kavitha, 2001), reducing rise in blood sugar, reduce the amount of insulin required (Amrithaveni and Thirumanidevi, 2004) and facilitate slow absorption of glucose along the passage through gastrointestinal tract. Bitter gourd - a fibre rich vegetable is used as an adjunct in the Management of diabetes mellitus. It was effective in preventing polyuria and polydypsia in diabetes (Shetty et al., 2005). Sustained pectin ingestion has positive effect on gastric emptying and glucose tolerance in non-insulin – dependent diabetic patients (Schwart, et al., 2004; Adams, 1999)

The cooked leaves of moringa (Drumstick leaves) were shown to decrease blood sugar levels (John and Anna, 2005). According to Amrithaveni and Thirumanidevi (2004) fenugreek seed powder in the diet reduces blood sugar and urine sugar with concomitant improvement in glucose tolerance and diabetic symptoms in both IDDM and NIDDM Patients.

Acute ingestion of small amounts of fructose can improve glucose homeostasis. HbA1C was significantly lower at 2 months. Subjects with NIDDM may benefit from daily supplementation of catalytic amounts of fructose in their diet. (Vaisman and Evaniv, 2006)
2.8.1. Low Glycemic Index Foods For Diabetic Mellitus

The glycemic index is defined as the blood glucose response of food stuff in comparison with glucose or some other standard, (e.g.) white bread. This index measures how much blood glucose increases in two or three hours after eating. The glycemic index ranks foods on how they affect blood glucose levels, and it is about the quality of the carbohydrates and not the quantity (Thilakavathy, 2008).

The Glycemic Index (GI) characterizes the carbohydrates in different food a better market of health effects than the sugar-starch or simple complex sugar distinction. Healthy foods with a low GI provide an additional benefit over and above that achieved by conventional low-fat foods in the management and prevention of diabetes and pre-diabetes. Many whole-grain products have a high Glycemic Index (Brand-Miller, 2007).

From controversial beginnings in the 1980s, the Glycemic Index has stood the test of time and scientific scrutiny. The recommendations of FAO/WHO (1998) and major diabetes associations, including Nutrition Subcommittee of The Diabetes Care Advisory Committee of Diabetes UK 2003, the Canadian Diabetes Association (2000), and Diabetes Australia (2001) refer to glycemic index. Most recently, the American Diabetes Association (2001) recognized that the use of the GI can provide an additional benefit over that observed when total carbohydrate is considered alone (Sheard et al., 2004).

Epidemiological studies using the NHANES II database and the 1986-87 British Adults survey data have shown a negative relationship between glycemic index and high-density lipoprotein cholesterol (Ford and Liu, 2002 and Frost et al., 1999). Also the data from Nurses Study and the Health Professional study have shown correlations between low-GI diets and decreased incidence of diabetes (Salmeron et al., 1997 and Salmeron et al., 1997) and risk of cardiovascular disease (Liu et al., 2002). A study from Italy reported that high dietary glycemic index was
related to increased breast cancer risk (Augustin et al., 2001) and similar results were seen with Nurses Cohort Study (Higginbotham et al., 2004).

Higginbotham et al., (2004) support the relationship of high GI diets with colon cancer. Studies by Brand-Miller, (2007) with Type II diabetes demonstrated that the use of a low-GI diet in the treatment of diabetes improved control by a significant decrease in HbA1C. Improvements in cardiovascular risk factors also have been demonstrated by Opperman et al., (2004).

Rizkalla et al., (2003) state that, diet with low glycemic index value improve the prevention of coronary heart disease in diabetic and healthy subjects. In obese or overweight individuals, low glycemic index meals increase satiety and facilitate the controlled food intake with beneficial effect on post pyramidal glucose and lipid metabolism.

According to Grylls and McKenzie, (2004), reducing dietary saturated fat and excess body weight may be useful means of improving glycemic control in older adults with diabetes. Increasing physical activity and reducing energy from dietary source may assist weight control, the former particularly in women.

A study on low glycemic index lunch on satiety in over weight and obese people with Type II diabetes, suggest the need to promote culturally based combined foods .with high fiber and low glycemic index. This approach might contribute to the prevention of obesity increasing the perception of satiety while also improving metabolic control of diabetes. In addition, this is a low cost approach for people with limited financial resources (Jimenez et al., 2005).

Daily incorporation of low glycemic index carbohydrates in meal planning can be an effective diabetes self management strategy for glycemic control and weight management. The documented responses to the subject's conceptual and practical knowledge of the glycemic index confirm their acceptance of this approach as a permanent behavioral life style change and not a "diet". Positive
results of this Study attest to what worked for these subjects inviting educators to consider offering low glycemic index dietary advice to their diabetic patients (Burani and Longo, 2006).

A study on glycemic index of commonly consumed Indian foods proved that boiled dry peas and boiled rajmah had significantly low glycemic index values while bajra, roti and cooked sago had significantly high glycemic index values in normal subjects. On the other hand, in diabetic subjects boiled form of rajmah, cowpea, lentil and whole Bengal gram had brought about significantly low glycemic responses. Hence boiled bengal gram can be advocated for consumption to the diabetic subjects due to their low glycemic indices (Nalwade et al., 2003).

Saroja, (2000) recommends that 60 to 65 percent of energy requirements be derived from the complex carbohydrates contained in cereals and pulses for the effective control of diabetes. Cereals in the form of rice and wheat along with vegetable proteins in the form of pulses and legumes increases the protein content of the diet and also substantially increase the soluble fibre content of the diet. This, results in improved peripheral sensitivity to insulin and the glycemic indices of food stuff are also lowered.

Fibre rich foods delay glucose absorption from the small intestine and thus reduce post pyramidal blood glucose concentrations. Studies by Mohan et al., (2003) indicate that by increasing the non-starchy vegetables containing 3 percent to 4 Percent of energy as carbohydrate (green leafy vegetables, cucumber, cauliflower, ladies finger etc.) in the diet, the bulk of the meal was increased and this increases the fibre content of the diet. Blood glucose response to the ingestion of carbohydrate containing foods has been shown to vary dramatically depending on factors including the molecular structure of the carbohydrate, fiber content and degree of processing (Ludwig, 2002). Powell, (2002) opines eating white bread results in two and a half times the increase in
blood sugar than eating the same amount of carbohydrate from barley or chick pea.

Long term dietary treatment with increased amounts of fiber rich low glycemic index natural foods improves blood glucose control and reduces the number of hypoglycemic events in Type I diabetic patients (Rosalba, 2000).

Low glycemic index diet enhances weight control since the diet promotes satiety, minimizes post pyramidal insulin secretion and maintains insulin sensitivity (Jannette et al., 2002).

Replacing foods with a higher glycemic index by those with lower glycemic index resulted in improved glycemic control and reduced fasting serum lipids. Various properties of foods such as particle size, botanical structure and properties of the starch influence the metabolic responses to carbohydrate foods (Javi et al., 1999).

Zhang, (2004) concluded that knowledge of glycemic index seems to be easily understood and accepted by diabetic subjects and is beneficial to dietary practice, blood glucose and lipids.

Dan Luckaczer et al., (2006) opine that cardiovascular disease is the leading cause of death in diabetic women. A group of 27 diabetic women were given a low glycemic index diet with 30g of soya protein per day for 12 weeks. Significant improvements were observed in ratios of total to high density lipoprotein cholesterol and of triacyl glycerol to high density lipoprotein cholesterol and blood pressure when compared with normal diet program.

Intake of whole grains and dietary fibre lower glycemic index and its food sources are associated with reduced risk of obesity and Type II diabetes (Edeiman, 2006).

Willett et al., (2002) states that a higher intake of cereal fiber has been consistently associated with lower diabetes risk. In diabetic patients, replacing high glycemic index carbohydrates with a low glycemic forms will improve glycemic control and, among persons treated with insulin, will reduce hypoglycemic episodes. These dietary changes can be made by replacing products made with white flour and
potatoes with whole-grain minimally refined cereal products, have also been associated with a lower risk of cardiovascular disease and can be an appropriate component of recommendations for an overall healthy diet.

Franz (2007) suggests that the total amount of carbohydrate in a meal is the primary meal-planning strategy for people with diabetes. The Glycemic index can be used as an adjunct for fine tuning of postprandial blood glucose responses. Kolset, (2003) quotes that glycemic index is developed to help persons with diabetes improve their blood glucose control in order to avoid long term complications. He also suggests that a carbohydrate diet with low glycemic index may reduce the risk of overweight, Type II diabetes and colon cancer. Also glycemic index could be used as a tool for dieticians and physicians for counseling persons with overweight and diabetes.

There is a large bulk of evidence that using low glycemic index (GI) foods has a very significant impact on the amelioration of metabolic disturbances observed in diabetic and hyperlipidemic patients. Improvement was observed not only in post pyramidal blood glucose and insulin variations but also in circulating plasma lipid levels and the morphology and function of adiposities. Slama et al., (2006) also suggests that the use of low glycemic index foods should be considered as one of the means and tools available to improve diabetes.

Burani and Longo, (2006) in their research study evaluated the incorporation of II w glycemic index carbohydrates into daily meal planning as an effective behavioral lifestyle change to improve glycemic control and weight management in diabetic patients. Low glycemic index medical nutrition therapy (LGI-MNT) counseling reduced $Hb_{A1C}$ by 19 percent and decreased BMI by eight percent. This was accomplished by the participants independently lowering the glycemic index values of their meals by 25 percent and they accepted this approach as a permanent behavioral lifestyle change and not a 'diet'.
2.8.2. Importance Of Dietary Antioxidants In Diabetes Mellitus

Antioxidant rich foods not only help to improve the serum antioxidant profile but also help to control blood sugar and serum lipid profile. There is a significant positive association with antioxidants and lifestyle diseases (Preethi and Nandini, 2005). According to Roginsky and Eduard, (2005) antioxidant defense has been recently recognized to originate from the chain - breaking antioxidant activity of natural polyphenol.

Many studies have pointed out that supplementation of antioxidant rich foods have positive impact on the diabetic subjects. Aliyu et al., (2005) reviews that consumption of fruits and vegetables is associated with reduced risk of free radical production. The potential protective effects of these foods may be due to their antioxidant vitamin contents. beta-Carotene one of the dietary sources of vitamin A, vitamin C and vitamin E are free radical scavengers and have been shown to quench singlet oxygen, superoxide, hydroxyl radical and peroxy radicals.

The intake of total antioxidants was significantly correlated with plasma lutenin, zeaxanthin and lycopene. Among individual food groups, coffee, tea, cocoa, wine, vegetables, fruits were significantly correlated. It supports that, dietary antioxidants other than well-known anti oxidants contribute to our antioxidant defense. The single greatest contributor to the total antioxidant intake was coffee (Svilaas et al., 2004).

Drinking green and black teas infusion can significantly lower the glucose level in blood by reducing the absorption of glucose. The polyphenols in tea inhibit the activity of alpha - amylase in saliva and reduce the activity of intestinal alpha-amylase, which in turn lowers the hydrolysis of starch to glucose and reduces glucose assimilation. Polyphenols can also decrease the activity of other digestive enzymes and reduce glucose absorption (Rana, 2005).
Diets rich in fruits and vegetables that are rich sources of antioxidant vitamins (Sablani et al., 2006) like beta - carotene, ascorbic acid and tocopherols, (Easwaran et al., 2002). It lowers the incidence of a number of diseases including Diabetes (Yochum, 2000). Boiling resulted in a drastic loss of catalase and peroxidase activity in leafy vegetables. A considerable proportion of potential antioxidant compounds are destroyed during boiling but some components exhibit thermo stability, which may be important in rendering protection (Padma and Anitha, 2005).

Cooking loss and Nutrient Retention Factors (USDA-Table of Nutrient Retention Factors 2003 http://www.nal.usda.gov/foodcomp/data) have already been evaluated in many studies. The antioxidant activity of spice extracts were retained even after boiling for 30 min at 100 degrees C, indicating that the spice constituents were resistant to thermal denaturation (Shobana and Naidu, 2000). Natural and synthetic anti-oxidants exert no effect on cooking yield and cooking loss. Vitamin E tested from natural antioxidant sources, reduced the formation of peroxides more effectively than the synthetic antioxidant like alpha tocopheryl acetate (Ayoola et al., 2011).

Beans are a good source of high quality protein, complex carbohydrates, dietary fiber, some vitamins and minerals. Beans also contain phytochemicals often considered as antinutritional factors, which include poly-phenols (condensed tannins and anthocyanins), protease inhibitors, lectins and phytic acid (Gonzalez et al., 2005).

Oranges play a vital role in preventing various diseases and maintaining good health and are rich in phytochemicals. Though they are renowned for their vitamin C content, they also contain about 170 elements, like flavonoids, phenolics, tannins and carotenes all of which play a crucial role in maintaining health in diabetes. They are rich source of dietary fibres (Dobriyal, 2005).

Oranges also contain folic acid, vitamin A, vitamin B, calcium and iron. An average fruit contains about 5 g of the vitamins. The
phenolic compounds present in oranges are known to have potent anti-inflammatory and anti-allergic actions. Orange flavonoids significantly reduce the risk of diabetes and cardiovascular diseases. They also play a crucial role in reducing the oxidative stress by their potent antioxidant properties. Oranges also help to repair the body tissues and speed up the healing process due to its rich vitamin C content (Dobriyal, 2005).

Vitamin E and C have proved to be potent antioxidants protecting lipids in plasma against oxidation. A combined intake of consumption of vitamin E and C together brought better results in blood lipid levels than consuming vitamin E or C alone. Hyperlipidemics and obese people should consume liberal amounts of fruits and vegetables that are rich in antioxidant vitamins to maintain blood lipid levels (Easwaran et al., 2002).

Curcuminoids - minor component present in turmeric possess antioxidant activity. Glutathione (gamma-glutamyl cysteinyl glycine) GSH is thought to be an important factor in cellular function and defense against oxidative stress and found that dietary GSH suppresses oxidative stress in vivo in prevention of diabetic complications such as diabetic nephropathy and neuropathy (Osawa and Joji, 2005).

The importance of natural antioxidants especially of plant origin has greatly increased in recent years (Chidamabaramurthy et al., 2002). Most studies have shown that synthetic antioxidants degrade cells over time and cause adverse health effect. Consumption of synthetic beta carotene increased risk of cancer. Synthetic vitamins have been shown to be treated like foreign substance in the body just as the drugs are. This means the body has to work hard to detoxify the body from them (Karen, 2007). Hence dietary antioxidants are today gaining high significance especially in controlling diseases like diabetes mellitus.
2.9. ANTIOXIDANT STATUS IN DIABETES MELLITUS

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals and these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions (Srinivas, 2005).

Antioxidants are substances that work in different ways to protect cells from biochemical damage (Preethi and Nandini, 2005). The most common antioxidant present in plant foods are carotenoids, phenolics, anthocyanins, flavonoids, flavones, ascorbic acid and vitamin E (Kaur et al., 2004). Plasma antioxidants can be decreased as compared to established normal values in abnormal or subnormal conditions, for instance as a consequence of disease related free radical production. Plasma antioxidants may be below the normal range due to insufficient dietary supply. Therefore the antioxidant profile can be used in conjunction with other parameters of oxidative stress status in case of stress related conditions like diabetes (Patel, 2002).

Antioxidants interrupt the signaling mechanism triggered by oxidants, thus playing a significant role in inter and intracellular signaling. Such antioxidants in large number exist as nutritive and non-nutritive form in food and aid in diminishing the reactive oxygen species effect (Srinivas, 2005).

According to Sesikaran, (2006), antioxidant systems help defend the body against reactive oxygen species but may be overwhelmed during periods of oxidative stress, which can cause lipid peroxidation, damage to DNA and cell death. Total Antioxidant Capacity (TAC) is the overall activity of antioxidants and antioxidant enzymes; and is used for monitoring and optimization antioxidant therapy. The depletion of TAC induced by oxidative stress is eliminated by release of stock organ antioxidants, mainly from liver.
and adipose tissue and the induction or activation of antioxidant enzymes (www.elsevier.com/locate/freeradbiomed).

2.9.1. Antioxidants In Diabetes Mellitus

Antioxidants have a major role in the prevention and control of diseases like diabetes mellitus. Free radicals having at least one unpaired electron that consume electron from other healthy molecules cause damage/oxidative stress to β cells in pancreas. Antioxidants, having one or more extra electrons donate an electron to a free radical; neutralize the free radical and oxidative stress (damage) in the cells (http://www.amazing-glutathione.com). Figure 2.4., shows the action of antioxidants neutralizing free radicals in human cell.

Figure 2.4. Antioxidants Neutralizing Free Radical In Human Cell

Oxygen being a reactive compound is capable of becoming damaging molecules termed as free radicals or Reactive Oxygen Species essentially possessing unpaired electrons. Oxidative destruction is caused by reactive oxygen species known as oxidants (e.g.: lipid peroxides such as hydrogen
peroxide) and free radicals (e.g.: superoxide, hydroxyl radical) (Veach, 2004).

The level of oxidative stress in diabetes mellitus is determined by the balance between the rate at which pro-oxidants are produced and the rate at which they are removed by antioxidant defense mechanism. Reactive oxygen species are normally produced in the body as part of oxygen metabolism or by UV radiation, nutritional deficiencies, bacterial, viral infections, toxic chemicals, xenobiotic metabolism, endocrine disorders like diabetes mellitus, cigarette or organic smoke exposure and some genetic diseases (Srinivas, 2005).

The excess free radicals circulating in the body oxidize the LDL, making them potentially lethal. The Reactive oxygen species can accelerate ageing process and have been linked to serious pathologies such as brain stroke, diabetes mellitus, cardio vascular diseases, hypertension, obesity (Panchnadikar et al., 2003) rheumatoid arthritis, Parkinson’s disease, Alzheimer’s disease and cancer (Campanella et al., 2006).

A deleterious effect of radiation is the production of reactive oxygen species, which result in damage to biomolecules. Free radicals and other reactive species are produced in the body primarily as a result of aerobic metabolism. Antioxidants and antioxidant enzymes exert synergistic actions in scavenging free radicals. Thus diabetics are much benefited (Fang et al., 2002).

Reactive Oxygen Species (ROS) are produced by oxidative phosphorylation, Nicotinamide Adenine Dinucleotide Phosphate Oxidase (NADPH), Xanthine oxidase, the uncoupling of lipoxygenases and glucose auto oxidation. Once formed, reactive oxygen species deplete antioxidant defenses, rendering the affected cells (β cells) and tissues more susceptible to oxidative damage. Reactive oxygen species are also important as second messengers in the regulation of intracellular signaling pathways and ultimately gene expression (Niedowicz and David, 2005).
The oxygen free radicals are part of the large family of Reactive oxygen species (ROS) i.e. they comprise species with a strong oxidizing tendency both of radical nature (the superoxide radical, the hydroxyl radical) and a non radical nature (ozone, hydrogen peroxide). Reactive oxygen species are continuously produced in the human body through a metabolic process, if not deactivated, these species, owing to their strong reactivity can in practice cause damage to all molecules, which can be inferred as a major cause for diabetes mellitus (Campanella et al., 2006).

Human body possesses defense mechanisms to protect cells against damage. Three defense strategies are used to protect cells from oxidative damage.

1. Neutralization of free radicals.
2. Metabolism of free radicals by enzyme.
3. Repair of macromolecular damage.

This is accomplished by water soluble antioxidants e.g.: ascorbic acid, cysteine, lipid soluble antioxidants (e.g.; tocopherol and retinols), specific enzymes and flavonoids (Veach, 2004).

### 2.9.2. Antioxidants and Oxidative Stress In Diabetes Mellitus

Oxidative stress induced by reactive oxygen species (ROS) is implicated in the pathogenesis of diabetes mellitus and a variety of disease (Kataja-Tuomola et al., 2011). The redox state is finely tuned to preserve cellular homeostasis through the expression of antioxidant enzymes and hence regulation of oxidants (Ziyatdinova et al., 2006). Oxidative stress is involved in the origin of type 1 diabetes (Chistiakov, 2004; Haskins et al., 2006). Diabetic (DM) patients are claimed to be under oxidative stress because of hyperglycemia. The influence of free radical production by this hyperglycemic induction may involve cardiovascular complications in diabetes (Likidililid et al., 2007).

Mammalian cells have a complex network of antioxidants like catalase, superoxide dismutase (SOD), reduced glutathione etc to
scavenge reactive oxygen species. Oxidative stress ensues when ROS evade or overwhelm antioxidants. Due to their highly reactive and non-specific nature, ROS can attack almost all biomolecules including lipid membranes and β cells (Kaur et al., 2008).

Lipid peroxidation is a process where pro-oxidant compounds such as reactive oxygen species react with poly unsaturated fatty acid of biological membranes (Veach, 2004). Lipid peroxides derived from the oxidation of polyunsaturated fatty acids of membranes and are capable of further lipid peroxidation by a free radical chain reaction (Kaur et al., 2008). In diabetes many factors raise blood lipid level, this is because carbohydrates and lipid metabolism are interrelated to each other; if there is any disorder in carbohydrate metabolism it also leads to disorder in lipid metabolism so there is high concentration of cholesterol and triglycerides and due to this there is reduction in HDL cholesterol levels (Smith, and Lall, 2008).

Oxidative stress leads to damage of all major molecules including DNA, proteins and lipids, and is often the precursor for many diseases such as diabetes, cancer, arteriosclerosis and the aging process (Kaur et al., 2008). Teksen et al., (2007) opines that if there is marked imbalance between the production and removal of reactive oxygen, then oxidative stress arises which aggravates diabetic complications.

Ziyatdinova et al., (2006) has cited that oxidative stress could stem from endogenous sources through normal physiological processes such as mitochondrial respiration or haemoglobin oxidation; and from exogenous sources such as exposure to pollutants, ionizing irradiation or other extreme factors.

Oxidative stress leads to the generation of reactive oxygen species (ROS) including free radicals. Their amount depends on generation rate and the antioxidant defense system of human body particularly of blood. ROS are strongly implicated in the pathophysiology of diseases such as diabetes mellitus, cancer, heart
diseases and atherosclerosis, aging, renal, inflammatory, infectious and neurological diseases (Kaur et al., 2008).

Diabetes mellitus can be better controlled if free radical reactions are inhibited by decreasing the level of active products from oxygen reduction or by removing the transition metals group (Fe, Cu) by their bounding with proteins. Free radicals are also eliminated from the body by their interaction with antioxidants. At a later phase of oxidative stress, the Total Antioxidant Capacity (TAC) falls due to depletion of antioxidants, this in turn aggravates diabetes mellitus. LMW antioxidants penetrate specific location in the cell where oxidative stress may occur and protect against ROS (Ziyatdinova et al., 2006). A significant increase of TAC occurs after supplementation with vitamins C, E, β-carotene and phenolics of green and black tea (Larini et al., 2004), and is found beneficial in diabetes mellitus (Anuradha and Vidhya, 2001; Jasim et al., 2011).

2.9.3. Classification of Antioxidants

Antioxidants are classified on the basis of various factors like function, molecular weight, mechanism of action and reactions, ability to reduce ROS, conversion of other oxidants.

According to Ziyatdinova et al., (2006) two classes of antioxidants are known: the low-molecular weight (LMW) compounds (tocopherols, ascorbate, betacarotene, glutathione, uric acid, bilirubin etc.) and the proteins (albumin, transferrin, caeruloplasmin, ferritin, superoxide dismutase, catalase, glutathione peroxidase etc.). LMW antioxidants penetrate specific location in the cell where oxidative stress may occur and protect against ROS. A significant increase of Total antioxidant capacity (TAC) really occurs after supplementation with vitamins C, E, and beta carotene and phenolics of green and black tea (Ghiselli et al., 2000).

Depending upon the mechanism of action, antioxidants are classified into two as radical chain breaking antioxidants and preventative antioxidants. Chain breaking antioxidants convert
reactive free radical to stable and thus non-aggressive molecules through hydrogen atom transfer reactions (Kaur et al., 2004).

Table 2.2., shows the classification of antioxidants by Sesikaran, (2006), where, antioxidants are classified into two groups:

(1) Antioxidants which provide a buffer against the oxidative pressure of reactive oxygen species on other molecules by their own amenability to oxidation and

(2) Other enzymes that catalyse the conversion of oxidants and reduce the presence of these free radicals.

<table>
<thead>
<tr>
<th>ENDOGENOUS</th>
<th>EXOGENOUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic</td>
<td>Non enzymatic</td>
</tr>
<tr>
<td>Se-glutathione peroxidase</td>
<td>Coenzymes Q 10</td>
</tr>
<tr>
<td>Superoxidedismutase Catalase</td>
<td>Feritin</td>
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<tr>
<td></td>
<td>Cerruloplasmin</td>
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</table>

(Source: Sesikaran, 2006)

**2.10. ENDOGENOUS – ENZYMATIC ANTIOXIDANTS**

Endogenous-Enzymatic Antioxidants are produced by the body and are not obtained from food sources; they are far more potent than exogenous antioxidants. Endogenous antioxidants repair *all* of the free radical damage by initiating cell regeneration from inside to outside, whereas, exogenous antioxidants only repair some of the free radical damage from the outside to inside by stimulating (not initiating) cell regeneration. There are five extremely powerful endogenous antioxidants. They are: Glutathione (GSH), Superoxide Dismutase (SOD), Catalase, Alpha Lipoic Acid (ALA) and Coenzyme Q10 (CoQ10). The production of endogenous antioxidants declines with age; glutathione levels decline about 10-15% per decade as we grow older. This decrease in endogenous antioxidants is found to be a strong factor in contributing to premature aging and degenerative diseases (http://www.amazing-glutathione.com).
Age-related health issues and degenerative diseases like Diabetes, Alzheimer's, Cancer, Heart Disease, and many other health concerns can be prevented by increasing the endogenous antioxidants at cellular levels. SOD, CAT and GPx constitute a mutually supportive team of defense against Reactive Oxygen Species (Nirmala et al., 2011).

2.10.1. Glutathione Peroxidase

Glutathione peroxidase was discovered in 1957 by Gordon C. Mills (Muller et al., 2007). The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases and glutathione S-transferases. Glutathione peroxidase (PDB 1GP1, 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. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Ran et al., 2007).

Glutathione is the Master Antioxidant as it does not need any other antioxidants to do its job. Glutathione is about 5,000 times stronger than any other antioxidant and has more "extra" electrons to share. For example: Vitamin E has 3 extra electrons to share, Vitamin C has 5 extra electrons to share, Oligomeric Proanthocyanidin (OPC) has 250 extra electrons to share, Superoxide Dismutase (SOD) has 10,000 extra electrons to share, Glutathione (GSH) has 1 Million extra electrons to share (http://www.amazing-glutathione.com).

Glutathione peroxidase1 (GPx1) is the most abundant version, found in the cytoplasm of nearly all mammalian tissues; with a substrate is hydrogen peroxide. Glutathione peroxidase2 is an intestinal and extracellular enzyme, while glutathione peroxidase3 is extracellular, especially abundant in plasma. Glutathione peroxidase4 (GPx4) has a high preference for lipid hydroperoxides; it is expressed in nearly every mammalian cell, though at much
lower levels. So far, eight different isoforms of glutathione peroxidase (GPx1-8) have been identified in humans (Muller et al., 2007).

Mammalian GPx1, GPx2, GPx3, and GPx4 have been shown to be selenium-containing enzymes, whereas GPx6 is a seleno protein in humans and cysteine-containing homologues in rodents. GPx1, GPx2, and GPx3 are homotetrameric proteins, whereas GPx4 has a monomeric structure. As the integrity of the cellular and subcellular membranes depends heavily on glutathione peroxidase, the antioxidative protective system of glutathione peroxidase itself depends heavily on the presence of selenium (Ran et al., 2007).

Muller et al., 2007 gives an example reaction that glutathione peroxidase catalyzes: \[ 2 \text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GS-SG} + 2\text{H}_2\text{O}, \]
where GSH represents reduced monomeric glutathione, and GS–SG represents glutathione disulfide. Glutathione reductase then reduces the oxidized glutathione to complete the cycle:

\[ \text{GS–SG} + \text{NADPH} + \text{H}^+ \rightarrow 2 \text{GSH} + \text{NADP}^+ \]

the low activity of glutathione peroxidase causes accumulation of \( \text{H}_2\text{O}_2 \).

Lack of Glutathione peroxidase1 (GPx1) enhances atherosclerosis among diabetics. GPx1 play a pivotal role in antioxidant defense and the progression of Diabetes Mellitus and associated atherosclerosis. GPX is a relatively stable enzyme, but it may be inactivated under conditions of severe oxidative stress. Inactivation of the enzyme may occur through glycation governed by prevailing glucose concentration (Lewis et al., 2007).

A study conducted by Likidlilid et al., (2007) found that the level of red cell glutathione peroxidase (GSH reduced monomeric glutathione) was significantly lower in type 1 diabetic patients compared to age-matched normal control. But the red cell GPx activity was significantly increased which may be due to the higher rate of consumption of GSH, increasing GPx activity or a reduction of pentose phosphate pathway, stimulated by insulin, resulting in lowered GSH recycle. A positive correlation correlation was found
between Fasting Plasma Glucose level (FPG) and GPx activity in type I diabetic patients compared with healthy normal subjects in diabetic patients.

2.10.2. Superoxide-dismutase

The enzyme superoxide dismutase (SOD, EC 1.15.1.1), catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. It is an important antioxidant defense in nearly all cells exposed to oxygen (Corpas et al., 2006).

SOD was discovered by Irwin Fridovich and Joe McCord, which were known earlier as several metalloproteins with unknown function (for example, CuZnSOD was known as erythrocuprein). Several common forms of SOD exist: they are proteins co-factored with copper and zinc, or manganese, iron, or nickel (Muller, et al., 2006).

The cytosols of virtually all eukaryotic cells contain an SOD enzyme with copper and zinc (Cu-Zn-SOD). The Cu-Zn enzyme is a homodimer of molecular weight 32,500. The two subunits are joined primarily by hydrophobic and electrostatic interactions. The ligands of copper and zinc are histidine side chains (Corpas et al., 2006).

E. coli and many other bacteria also contain a form of the enzyme with iron (Fe-SOD); some bacteria contain Fe-SOD, others Mn-SOD, and some contain both. Fe-SOD can be found in the plastids of plants. The active sites of Mn and Fe superoxide dismutases contain the same type of amino acid side chains (Muller et al., 2006).

In humans, three forms of superoxide dismutase are present. SOD1 is located in the cytoplasm, SOD2 in the mitochondria and SOD3 is extracellular. The first is a dimer (consists of two units), while the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, while SOD2 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). SOD
outcompetes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity (Groner, 1994).

Superoxide dismutase 1 soluble (SOD1), which is a human protein and gene encodes one of three forms of the human superoxide dismutase. SOD1 which binds copper and zinc ions is one of two isozymes responsible for destroying free superoxide radicals in the body. The encoded isozyme is a soluble cytoplasmic protein, acting as a homodimer to convert naturally occurring, but harmful, superoxide radicals to molecular oxygen and hydrogen peroxide (Elchuri et al., 2005; Muller et al., 2006).

The major protector against superoxide anion in the extracellular space is extracellular-superoxide dismutase (EC-SOD). EC-SOD is the major SOD isozyme in plasma and forms an equilibrium between the plasma phase and heparan sulfate proteoglycan on the surface of the endothelium (Adachi et al., 2007). The reference value established by Bogdanska et al. (2003) for superoxide dismutase-absolute activity is 122.4-333.9±2SD U/ml and for specific activity is 884.2-2119±2SD U/gHb.

Superoxide is one of the main reactive oxygen species in the cell that serves as a key antioxidant. Mice lacking SOD2 die several days after birth, amidst massive oxidative stress, develop a wide range of pathologies, including hepatocellular carcinoma, an acceleration of age-related muscle mass loss, an earlier incidence of cataracts and a reduced lifespan. Mice lacking SOD3 do not show any obvious defects and exhibit a normal lifespan (Li et al., 1995)

Overexpression of SOD1 has been linked to Down's syndrome (Muller et al., 2006). Superoxide dismutase is effective as a nutritional supplement when bound to the polymeric films of wheat matrix gliadin (a delivery method also known as glisodin). Gliadin is an ideal carrier because it protects SOD from stomach acid and enzymes found in the digestive system which break down its molecular structure. This has been established in a variety of animal studies and human clinical trials, in which SOD's generally high
antioxidant capacity is kept intact under a variety of conditions (Groner, 1994).

2.10.3. Catalase

Catalase is a common enzyme found in nearly all living organisms that are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. Catalase has one of the highest turnover numbers of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second (Chelikani et al., 2004).

Catalase was first noticed as a ‘substance’ in 1818 when Louis Jacques Thénard, who discovered H$_2$O$_2$, suggested that the breakdown of H$_2$O$_2$ is caused by a ‘substance’. In 1900, Oscar Loew was the first to give it the name catalase, and found its presence in many plants and animals (Loew, 1900).

Hydrogen peroxide is a harmful by-product of many normal metabolic processes: to prevent damage, it must be quickly converted into other less dangerous substances. Catalase is frequently used by cells to decompose hydrogen peroxide into less reactive gaseous oxygen and water molecules (Chelikani et al., 2004).

High concentrations of hydrogen peroxide may damage heme proteins, cause cell death, and together with redox active metal ions, produce highly toxic hydroxyl radicals. Catalase can also oxidize different toxins, such as formaldehyde, formic acid, phenols, and alcohols; during these processes hydrogen peroxide is transformed (Robertson et al., 2003).

Catalase is the main regulator of hydrogen peroxide metabolism, (Mueller et al., 1997). Hydrogen peroxide has novel insulin-like effects, e.g., inhibition of lipolysis and reactivation of phosphoenol pyruvate carboxy kinase (Visentin et al., 2003; Sutherland et al., 1997) and insulin moderates hydrogen peroxide generation and catalase synthesis. H$_2$O$_2$ has been reported to
damage pancreatic β-cells and inhibit insulin signaling (Pomytkin and Kolesova, 2003).

Catalase is important in antioxidant defense against hydrogen peroxide (Robertson et al., 2003; Hansen, 1999), but there are conflicting reports on decreases (Bloch et al., 2003; Sindhu et al., 2004), increases (Weidig et al., 2004), and no change (Manea et al., 2004) in catalase activity in diabetic patients. But many studies shows that, an elevated concentration of hydrogen peroxide, due to decreased catalase activity, could contribute to oxidative destruction of pancreatic β cells, to decreased insulin secretion and insulin effectiveness, and to the onset of diabetes. Dysregulation of catalase synthesis could be responsible for decreased blood catalase in gestational diabetes and its change in the second and third trimester (Góth et al., 2005; Chistiakov 2004).

The reference value established by Bogdanska et al., (2003) for Catalase-absolute activity is 17.9-41.1 ±2SD k and for specific activity is 118.8-222.0 ±2SD k/g Hb. The reference range for blood catalase activity according to Góth and Vitai (1996), was 113.3 ± 16.5 MU/l. Decreased blood catalase activity was defined as <80.3±2SDMU/l.

According to recent scientific studies, low levels of catalase may play a role in the greying process of human hair. Hydrogen peroxide is naturally produced by the body and catalase breaks it down. If there is a dip in catalase levels, hydrogen peroxide cannot be broken down. This causes the hydrogen peroxide to bleach the hair from the inside out. Scientists believe this finding may someday be incorporated into anti-greying treatments for aging hair (Hitti, 2009).

Today catalase is widely used; for contact lens hygiene, in cosmetics industry to increase cellular oxygenation in the upper layers of the epidermis, in textile industry for removing hydrogen peroxide from fabrics, in food industry for removing hydrogen peroxide from milk prior to cheese production, in food wrappers to
2.1. EXOGENOUS–NON-ENZYMATIC ANTIOXIDANTS

Exogenous – Non-Enzymatic antioxidants are obtained from diet by eating antioxidant-rich foods and by taking supplements. Some well-known examples of exogenous antioxidants are vitamins A, C, and E. Even though exogenous antioxidants can be obtained from food sources, in our modern day world it is nearly impossible to get enough exogenous antioxidants from our diet to neutralize all of the free radicals generated; hence antioxidant supplementation is so vital.

2.1.1. Ascorbic Acid (Vitamin C)

Ascorbic acid or "vitamin C" is a monosaccharide oxidation-reduction (redox) catalyst found in both animals and plants. This redox catalyst can reduce, and neutralize, reactive oxygen species such as hydrogen peroxide. In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the redox enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants (Padayatty et al., 2003). Water soluble antioxidants like vitamin C and glutathione scavenge reactive oxygen species in fluid outside the cell and within the cell. In eye, vitamin C has been shown to reduce lipid peroxide damage. (Veach, 2004)

Vitamin C was found to account for 65-100% of the antioxidant potential of beverages derived from citrus fruits but less than 5% in apple and pineapple juice (Gardner et al., 2000). According to Kaur et al., (2004) during processing of tomato juice vitamin C is oxidized. Ascorbic acid content of the legumes was elevated on soaking and germination. To minimize losses of ascorbic acid it is preferable to cook legumes for shorter period as ascorbic acid is heat sensitive (Sharma et al., 2002).
2.11.2. Tocopherol (Vitamin E)

The free radicals are usually removed or inactivated in vivo by a team of antioxidants Vitamins. The most outstanding contributions to the epidemiology of the effects of dietary supplementation with vitamin E have been studied extensively, which showed that a 40% reduction in coronary artery disease is seen in those individuals who supplemented their diet with vitamin E intake (Bansilal et al., 2007).

Vitamin E, a lipid soluble antioxidant (Veach, 2004) is extensively distributed in wheat germ, sunflower seed, safflower seed, corn and soyabean. Vitamin E has 8 different stereoisomer of which alpha-tocopherol is known to have greatest biological activity and act as a potent inhibitor of lipid peroxidation both invitro and in vivo. Impairment in the antioxidant system in the erythrocytes could be ameliorated by dietary vitamin E supplementation (Devasena et al., 2002).

Vitamin E, a chain braking antioxidant stored in cell membranes and mitochondrial membrane prevents endogenous mitochondrial production of reactive oxygen species. Vitamin E, C and Glutathione are the three principal antioxidants in metabolism relevant to glaucoma (Veach, 2004). Dietary fat is required for the absorption of vitamin E. The amount of dietary fat in a meal and the food matrix of the meal influence the absorption and the bioavailability of Vitamin E (Jeanes et al., 2004). Vitamin E includes 4 tocopherols and 4 tocotrienols which differ in their biological and antioxidant activity. Alpha-tocopherol has the highest biological and antioxidant activity. A high level of PUFA in the diet causes an increase in the susceptibility of tissues to lipid oxidation, increasing the requirement for Vitamin E (Cortinas et al., 2004).

Vitamin E intake was significantly associated with a reduced risk of type 2 diabetes. Intake of alpha tocopherol, gamma tocopherol, and delta tocopherol and beta tocotrienol was inversely related to a risk of type 2 diabetes. The study supports the
hypothesis that development of type 2 diabetes may be reduced by the intake of antioxidants in the diet (Montonen et al., 2004).

Vitamin E and selenium function synergistically in the myocardium to provide important antioxidant defenses in iron-overload states including increased concentrations of selenium, increased glutathione peroxidase activity and decreased concentrations of iron (Burt et al., 2000). According to Rube and McDonald, (2001) vitamin E and lipoic acid supplements lessen the impact of oxidative damage caused by dysregulation of glucose metabolism.

2.11.3. Vitamin A

Vitamin A is a fat-soluble antioxidant, which is essential for growth, maintenance of visual function, reproduction and differentiation of epithelial tissue. Carotenoids are known to quench oxidant species such as singlet molecular oxygen; the mechanism seems to be a physical quenching reaction that does not affect chemically the structure of the pigment (Ray and Husain, 2002). The normal range for serum vitamin A has been reported as 30 to 60 µg/100 ml in the U.S. and 20 to 50 µg/100 ml in England. It may be assumed that there is no deficiency when the serum level is above 20 µg/100 ml. Serum concentration below 10µg/100ml shows impaired light-dark adaptation (Winsten and Dalal, 1972).

A new study by the Agricultural Research Service indicates that high levels of Vitamin A could suppress the development of type 1 diabetes amongst laboratory mice (http://www.diabetes.co.uk/news/2008/Apr/type-1-diabetes-suppressed-by-vitamin-a.html).

According to an animal study by American scientists high levels of vitamin A may curb the onset of type 1 diabetes by protecting against the attack of insulin-producing beta cells. Therefore dietary intervention with foods or food constituents may prove to be beneficial in the prevention and/or management of type 1 diabetes. Increasing polyphenol or vitamin A levels in the diet may have profound effects on suppressing inflammatory immune cells.
and reducing the oxidative damage in the islets that contributes to loss of beta cells (Zunino et al., 2007)

2.11.4. Carotenoids

Carotenoids are natural pigments which are synthesized by plants and are responsible for the bright colors of various fruits and vegetables. There are several dozen carotenoids in the food that we eat, and most of these carotenoids have antioxidant activity. Beta-carotene is the most common carotenoid in fruits and vegetables (Paiva and Russell, 1999).

Beta-carotene is viewed as a non-toxic precursor of vitamin A. It functions as an efficient singlet oxygen quencher and as a radical trapping antioxidant at low oxygen pressure to reduce the extent of nuclear damage and to inhibit lipid peroxidation (Yang et al., 2004). Mixtures of carotenoids or associations with other antioxidants (e.g.: vitamin E) can increase their activity against free radicals. Carotenoids (including beta-carotene) can promote health when taken at dietary levels, but may have adverse effects when taken in high dose by subjects who smoke or who have been exposed to asbestos (Paiva and Russell, 1999). According to Ray and Singh, (2003) annual production of carotene by nature amounts to about 10,000 tones. Higher rate of biosynthesis of carotenoids occur in the peel than in the flesh of fruits.

Dietary carotenoids are an important source of vitamin A for normal metabolic and physiological functions. A number of factors may affect the bioavailability of provitamin A and carotenoids; such as their conversion to vitamin A, rate of absorption, transport, chemical nature and fat content of the diet. The bio-availability of beta-carotene from vegetables in particular has been reported to below 14% from mixed vegetables (Gireesh and Nair, 2004).

High protein in the diet increased the levels of antioxidant enzymes, catalase and superoxide dismutase (SOD) and that excess vitamin A supplementation functions synergistically with
high protein in diet to increase antioxidant enzymes level. (Karar et al., 2002)

Plant foods containing carotenoids are present in dark green leafy vegetables, yellow and orange coloured fruits. Among the traditional foods Green Leafy Vegetables (GLV) are readily available and form the cheapest sources of carotene. Dehydrated vegetables are concentrated source of carotenes. Loss of carotenoids by sun drying was 74% of total carotene. 90% of carotene retention obtained in freeze drying and microwave drying and hence these methods could be considered as best methods of dehydration for retention of carotenes (Chandra et al., 2001).

Tomato is a rich source of carotenoids, but it contains only small amounts of flavonoids in its peel. Increasing the flavonoid content will improve the overall nutritional qualities of tomatoes. (Prabhakaran, 2003)

2.11.5. Selenium

Selenium having antioxidant support function is a crucial nutrient for maintaining human health in diabetes and was discovered by Berzelius in 1817. Deficiency of selenium in animals makes them susceptible to injury by certain types of oxidative stress. At least one human disease occurs in selenium deficient individuals. The plasma or serum selenium concentration is often used to assess selenium nutritional status. A plasma selenium concentration of 8 micrograms/dl or greater in a healthy subject indicates that plasma selenoproteins are optimized and the subject is selenium replete. The Third National Health and Nutrition Examination Survey determined plasma selenium in 17,630 subjects in the US and results indicate that more than 99% the subjects studied were selenium replete (Raymond, 2002).

Selenium is the major selen compound in cereal grains and enriched yeast. It is a component of a number of important
selenoproteins and enzymes (Raymond, 2002) required for such functions as tissue damage, immune response (Sesikaran, 2006) antioxidant defense, reduction of inflammation, thyroid hormone production, DNA synthesis, fertility and reproduction. (Raymond, 2002). The use of high-selenium east can raise the selenium status in human body (Rayman, 2004).

Blood or plasma levels of selenium are usually lower in patients with cancer than those without this disorder. Selenium content of plants is depended upon the region of growth. Selenium reduces the incidence of diabetes and tumours. The recommended daily allow once for selenium is 55µg/day for both men and women (Food and Nutrition Board, Institute of medicine 2000). FAO/WHO (2002) recommends 26µg/day for women and 34µg/day for men (Whanger, 2004).

Food is the major source of selenium with drinking water and air being minor contributors. Dietary intake varies with the geographical source of the foods and the eating habits of the local populations. Fish and organ meats are richest sources followed by muscle meats, but the bioavailability of selenium is greatest from selenium-enriched yeast, cereals and grains. Dairy products, fruits and vegetables tend to be the poorest sources of selenium. The main sources are sea food, poultry and eggs and to a lesser extent other muscle meats. The contribution of cereals to dietary selenium intake varies with the source of the crop (Thomson, 2004).

Trace elements and vitamins that support antioxidant function, particularly high dose parenteral selenium either alone or in combination with other antioxidants are safe and may be associated with a reduction in diabetic mortality in critically ill patients. Improving selenium status could help protect against tissue damage and infection in critically ill adults (Sesikaran, 2006).
2.11.6. Copper

Copper is an essential trace metal which plays a fundamental role in the biochemistry of the human nervous system. Ceruloplasmin contains 95% of the copper found in human plasma (Waggoner et al., 1999). In rats, copper deficiency leads to low copper metalo enzyme activity, high serum cholesterol and cardiovascular lesions (Jones et al., 1997).

The amount of daily copper requirements for adults has been reported to be 1.28-2.5 mg/day. 10g of cocoa is sufficient to treat copper deficiency (Wakugami et al., 2001). Bioavailability of copper from human milk is high, were as it is lower from cow’s milk. Protein sources, amino acids, carbohydrates and ascorbic acid can affect copper availability; where as, phytate, zinc and iron appear to have little influence on copper absorption (Lonnerdal, 1996)

2.11.7. Zinc

Antioxidants play a critically role in keeping skin healthy. Zinc is an essential element of more than 200 metalloenzyme, including the antioxidant enzyme, superoxide dismutase and affects their conformity, stability, and activity. Topical zinc, in the form of divalent zinc ions has been reported to provide antioxidant photo protection for skin. Zinc ions may replace redox active molecules such as iron and copper at critical sites in cell membranes and proteins, alternatively and may induce the synthesis of metallothionine, sulfahydryl rich proteins that protect against free radicals (Rostan et al., 2002).

Zinc can protect the membranes from iron initiated lipid oxidation by occupying negatively charged sites with potential iron binding capacity. Zinc is a pivotal component of the antioxidant defense network that protects membranes from oxidation (Zago and Oteiza, 2001).

Zinc protects against UV radiation, enhances wound healing, contributes to immune and neuropsychiatric functions,
and decreases the relative risk of diabetes, cancer and cardiovascular disease. It is required for the normal growth, development and function of mammals. Zinc also is important for the proper functioning of the immune system, and for glandular, reproductive and cell health (Rostan et al., 2002).

2.11.8. Iron

Iron binding antioxidant capacity is decreased in diabetes mellitus. Redox active iron is known to play an important role in catalyzing peroxidation reactions (Van campenhout, 2006). The hemoglobin levels were significantly lower in the diabetics than in the non-diabetic groups and anaemia tends to occur earlier and is more severe in patients with diabetic than in an equivalent non-diabetic patient group (Ravanon et al., 2007).

The Specific activities of the enzymatic antioxidants like superoxide dismutase and catalase are expressed in terms of the level of hemoglobin concentration in the body. A reduction in iron stores and a calculated decrease of hemoglobin had the increasing effect on iron absorption (Hallberg, 2002). A diet rich in easily available iron is important for covering basal iron losses, menstrual iron losses and the high iron requirements for growth (Hallberg and Lena, 2002).

Bioavailability of iron from the dietary source depends on the iron content of the diet, actual composition of the diet and the absorption rate. The amount of dietary iron that is absorbed and utilized in the body for specified functions is determined by the bioavailability of iron from the diet, which in turn is dependent on the form of dietary iron and other constitutions of the diet. The absorption of haem iron which is derived from meat and flesh food is better while that of non haem iron derived from cereals, pulses, vegetables and fruits (i.e.) all foods of vegetarian origin is quite low. The absorption of haem iron is enhanced by increasing the ascorbic acid and beta carotene content of the diet. Addition of ascorbic acid rich foods like coriander leaves, drumstick leaves, agathi leaves,
cabbage and potato had increased the percentage of iron bioavailability (Sathya et al., 2002).

### 2.11.9. Flavonoids

Flavonoids are secondary compounds which occur ubiquitously in higher plants. They serve as pigments, UV protectants, defense agents against pathogens and signaling compounds in the interaction with beneficial micro-organisms. Flavonoids are also an integral part of the human diet with especially high concentrations in vegetables, fruits, tea and red wine (Prabhakaran, 2003). Ingestion of dietary polyphenols e.g.: from wine, cocoa or tea, improves endothelial dysfunction and lowers the susceptibility of LDL lipids to oxidation. Polyphenols affect endothelial function not solely as antioxidants but also a modulatory signaling (Sies et al., 2005). Flavonoids are potent antioxidants and epidemic studies indicate that high flavonoid intake is correlated with decreased risk of lifestyle diseases like diabetes and cardiovascular diseases (Kaur et al., 2008).

Tea and coffee are rich in polyhydroxylated phenolic compounds (polyphenols) including phenolic acids and flavonoids. Tea contains primarily flavonoids as well as up to 15% (25-50mg/cup). Coffee polyphenols are almost entirely chorogenic acid (100-200mg/cup). Polyphenols have a range of activities that might have a positive impact on human health. Higher intake of flavonoids is associated with lower risk for diabetes and chronic diseases (Hodgson et al., 2004).

According to Singh et al., (2003) two classes of compounds namely flavonoids and limonoids are present in citrus fruits and it is responsible for the bitterness. The fruits containing high flavonoids are bitter even when consumed as fresh. The peel of the citrus fruits contains very high amounts of flavonoids which make them highly bitter (Kaur et al., 2008) Table 2.3., shows the flavonoid content of different foods.
<table>
<thead>
<tr>
<th>Source</th>
<th>Flavonols</th>
<th>Flavones</th>
<th>Flavonones</th>
<th>Flavonols</th>
<th>Anthocyanins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rich source (&gt;10mg/100g)</td>
<td>Broccoli, Onion</td>
<td></td>
<td>Grapes, Oranges.</td>
<td>Tea, Wine Beverage,</td>
<td></td>
</tr>
<tr>
<td>Moderate source (4.0-9.9mg/100mg)</td>
<td>Turnip greens</td>
<td></td>
<td></td>
<td>Apples, Pears, Apricots, Peaches, Straw-berries</td>
<td></td>
</tr>
<tr>
<td>Low source (0.01-.99mg/100g)</td>
<td>Brussels sprouts, Cabbage, Cauliflower Lettuce, Spinach, Tomatoes, Potatoes, Green Beans, Kidney beans, Peas, Peanuts, Apples, Apricots, Grapes, Peaches, Pears, Oranges, Strawberry, Tea, Wine, Beer, Coffee.</td>
<td>Carrots, Spinach, Grape, Fruit, Oranges, Tea.</td>
<td></td>
<td>Carrots, Kidney beans, Lima beans, Apples, Nectarines, Peaches, Pears, Wine.</td>
<td></td>
</tr>
<tr>
<td>Possible Source: Specific levels not reported in literature</td>
<td>Corn, Squash, Lentils, Bananas, Nectarines.</td>
<td>Rice, Beer</td>
<td></td>
<td>Bananas</td>
<td></td>
</tr>
</tbody>
</table>

Source: (Prabhakaran, 2003)
Among the flavonoids, naringin is the widely occurring bitter substance. Tomatoes contain small amount of phenolic acid considering their high consumption in Indian diet they may constitute an important source of dietary phenolics (Kaur et al., 2008). Soybean is a major source of protein and also isoflavones to large parts of the world's population. There are 3 types of isoflavones in soybean and each type exists in 4 different chemical forms. Many studies on soybean supplementation and effect on diabetic and hyperlipidemic subjects have been done and found to have beneficial effects. Fermentation, heat treatments, chemical and enzymatic hydrolysis were reported to induce changes in composition of isoflavone profile (Zhu et al., 2005). Prabhakaran, (2003) reported that amount of isoflavones in dry soybean ranges from 1600-2400 mg/Kg. Soybean oil contains no isoflavones and soy sauce has little or no isoflavones.

High consumption of both flavonoids and isoflavones by Japanese women may contribute to their low incidence of diabetes and coronary heart disease compared with women in other countries. The major source of flavonoids was onions and that of isoflavones was tofu (Arai et al., 2001).

2.11.10. Phytosterols

Phytosterols or plant sterols lower the serum cholesterol concentration by competing with dietary and biliary cholesterol for intestinal absorption (Clifton et al., 2004, Patel and Paul, 2006). Phytosterols are found beneficial to diabetic subjects also. Sesame seed and wheat germ had the highest total phytosterol content (400-413 mg/100g) and Brazil nuts the lowest (95 mg/100 g) (Philips et al., 2005)

Phytosterol contained in vegetable oils is known to exert a hypoglycaemic and hypocholesterolemic function. It also suppressed the oxidation and consumption of alpha tocopherol in liposomal membranes. Phytosterol chemically acts
as an antioxidant, a modest radical scavenger and physically a stabilizer in the membranes (Yasukazu and Etsuo, 2003).

2.11.11. Lycopene

Lycopene can be considered as the vitamin of the 21\textsuperscript{st} century because of its significant physiological effect on human diet. Tomatoes are rich in lycopene, an antioxidant with immunostimulatory properties and contain moderate amounts of beta carotene, vitamin C and phenolics. Flavonoids and phenolic acids seem to be more concentrated in skin than flesh and vitamin E be coated in seeds. Lycopene content increases as tomatoes mature. Lycopene exhibits the highest antioxidant activity which may contribute to a reduction in disease risk. Consumption of tomato based foods may reduce the susceptibility of lymphocytes to oxidative damage. Lycopene is lost during dehydration. (Kaur \textit{et al.}, 2004)

Tomatoes are a source of antioxidants including vitamin C, total phenolics and carotenoids, particularly lycopene in the diet. Typical home processing of tomatoes leads to the loss of some antioxidant properties and change of color. Boiling and baking have a relatively small effect on ascorbic acid, total phenolics, lycopene and total antioxidant activity, while frying significantly reduces their important nutrients. The total lycopene content of fresh tomato was 37.7 mg/100g (Kerchofs \textit{et al.}, 2005).

2.12. SPIRULINA, THE GREEN GOLD

Spirulina, now named Arthrospira, is a microscopic and filamentous Cyanobacterium (blue-green alga) that has a long history of use as food. It belongs to the Oscillatoriaceae family, characterized by Spiral shaped chains of cells enclosed in a thin sheath (Belay, 2002). There are 35 species of spirulina with \textit{s.platensis} and \textit{s.maxima} as the most commonly used. The microscopic appearance of the algae is shown in Plate 2.1.
Scientists discovered that this photosynthetic life form was designed by nature 3-4 billion years ago, the very catalyst that enabled higher life forms to evolve by producing the oxygen needed in the Earth's atmosphere (www.spirulina.org.uk). This microscopic alga has successfully survived and renewed it ever since it was formed. There are reports that it was used as food in Mexico during the Aztec civilization some 400 years ago. It is still being used as food by the Kanembu tribe in the Lake Chad area of the Republic of Chad where it is sold as dried bread called “dihe” (Belay, 2002).

Spirulina are free-floating filamentous cyanobacteria characterized by cylindrical, multicellular trichomes in an open left-hand helix. Spirulina occurs naturally in tropical and subtropical lakes with high pH between 8 and 11 and high concentrations of carbonate and bicarbonate. S.platensis occurs in Africa, Asia and South America, whereas S.maxima is confined to Central America (http://en.wikipedia.org.spirullina).

Spirulina has been produced commercially for the last 20 years for food and specialty feeds. Commercial algae are normally produced in large outdoor ponds under controlled conditions. Some
companies also produce directly from lakes. Current production of Spirulina worldwide is estimated to be about 3,000 metric tons (www.spirulina.com).

The United Nations world food conference declared spirulina as “the best food for tomorrow”, and it is gaining popularity in recent years as a food supplement (Layam and Chandra 2006). Spirulina is rich in protein, vitamins, essential amino acids, minerals, and essential fatty acids. Spirulina is 60-70% protein, 5-10% vitamins especially, vitamin B\textsubscript{12}, provitamin A (β-carotene), and 3-5% minerals especially, iron. One of the few sources of dietary Gama-linolenic acid (GLA), it also contains a host of other phytochemicals that have potential health benefits (Belay, 2002).

Most cultivated spirulina is produced in open-channel raceway ponds, with paddle-wheels used to agitate the water. Current world production of spirulina for human consumption is more than one thousand metric tons annually. The largest commercial producers of spirulina are located in the United States, Thailand, India, Taiwan, China, Pakistan and Myanmar

http://en.wikipedia.org.spirullina

2.12.1. Harvest and Post-harvest Technology of Spirulina

Spirulina, a microscopic fresh-water micro-alga, is grown in a controlled high-sunshine, naturally warm, clean environment, well away from the pollution of densely-populated zones. The Culture of Spirulina from the ponds is pumped to Pre-filtration Unit for removing any matter of bigger size than Spirulina (www.spirulina.org.uk). The culture of spirulina in the open-channel raceway ponds is shown in Plate 2.2.

The Pre-filtration Unit comprises of series of specially selected filters to remove any extraneous matter. Then Spirulina is concentrated over harvesting filters and tiny Spirulina cells along with nutrient rich medium is recycled to ponds (www.spirulina.org.uk).
Bio-mass can be harvested by three methods namely- Flocculation, Centrifugation and Filteration. For flocculation the commonly used salts are Ferric chloride (FeCl₃), Aluminium Sulfate (Al₂(SO₄)₃), alum and Ferric sulfate (Fe₂(SO₄)₃). Centrifugation involves high energy consumption though the biomass recovery is rapid hence it has less used for commercial purposes. Filteration is carried out by filter pressing the biomass under pressure using pressure filters or by vaccum presses using rotary drum vacuum (Molina et al., 2003).

The harvested biomass slurry must be processed rapidly, or it can spoil within a few hours in a hot climate. Absence of water in the biomass enhances the recovery of nutrients. Different drying methods used are- spray drying, cross flow drying, sun drying, recirculatory hot air drying or freeze drying (Anonymous, 2003).

Concentrated Spirulina Biomass is thoroughly washed with clean demineralised water to remove any excess salts and tiny extraneous matter. The Spirulina Biomass slurry is immediately dried to retain its natural freshness. Total journey time of Spirulina Ponds to dry powder is 20 minutes. Spirulina dried into fine powder is immediately hermetically vacuum-packed into 20 to 50kg bags.
Spirulina bottles are often nitrogen-flushed prior to filling to minimise oxygen, thereby preserving the spirulina to provide low degradation and maximum shelf life. They are sealed with pressure-sensitive seals (http://www.synergynatural.com/spirulina.html).

Figure 2.5. Spirulina Manufacturing Process

Source:- http://www.synergynatural.com/spirulina.html

Pond Management is the most important factor in maintaining quality. Ponds are replenished with fresh nutrients after every harvest. Concentration of individual nutrients of pond medium is tested regularly in laboratory for time to time. The finished product is sampled and tested for various Physical, Chemical & Microbiological parameter according to norms of statutory bodies in Quality Control Department (http://herbaldistribution.com/spirulina).

The United Nations World Food Conference in 1974 lauded Spirulina as the 'best food for the future'. Recognizing the inherent potential of Spirulina in the sustainable development agenda, several Member States of the United Nations came together to form an intergovernmental organization by the name IIMSAM-
(Intergovernmental Institution for the use of Micro-algae Spirulina Against Malnutrition). IIMSAM aspires to build a consensus to make Spirulina a key driver to eradicate malnutrition, achieve food security and bridge the health divide throughout the world. 

http://en.wikipedia.org.spirullina

Spirulina has been proposed by both NASA (Controlled Ecological Life Support System) and the European Space Agency (MELISSA) as one of the primary foods to be cultivated during long-term space missions (www.spirulina.com). In 1967, Spirulina was established as a “Wonderful future food source” in the International Association of Applied Microbiology (Sasson, 1997).

2.12.2. Nutritional Profile of Spirulina - Antioxidant Potential

Oxidative stress may induce insulin resistance in peripheral tissues and impair insulin secretion from pancreatic β-cells. Antioxidants are suggested to decrease the risk of diabetes through reduction of oxidative stress (Kataja-Tuomola et al., 2011). Spirulina, the microalgae (Spirulina platensis, Spirulina maxima, Spirulina fusiformis) is considered as a valuable food source of antioxidants, macro and micronutrients including high quality protein, iron, gamma-linolenic fatty acid, carotenoids, vitamins B1, B2 and other antioxidants. During its cultivation in open reservoirs and in closed photo-bioreactors its biomass is enriched with trace elements such as iron, iodine, selenium, zinc, copper, manganese and chromium in bioavailable form and is a bioactive food supplement (Mazo et al., 2004).

Spirulina is 50-70% protein by weight containing essential amino acids – leucine, valine, isoleucine tryptophan, methonine, phenylalanine, threonine and lysine; and non essential amino acids like aspartic acid, glutamic acid alanine, arginine and glycine. The protein denatures at and above 67°C, in neutral aqueous solution (Cohen, 1997). It also contains carbohydrates like rhamnose,
fructose, ribose, mannose (Shekaram et al., 1987). Table 2.4. compares the nutrient composition of spirulina with egg and milk.

Table 2.4. Comparison of the Nutrient Composition of Spirulina with Egg and Milk

<table>
<thead>
<tr>
<th>Contents</th>
<th>Spirulina 10g</th>
<th>Egg 100 g</th>
<th>Milk 100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>6.5 g</td>
<td>13.3 g</td>
<td>3.2 g</td>
</tr>
<tr>
<td>Fat</td>
<td>0.5 g</td>
<td>13.3 g</td>
<td>4.1 g</td>
</tr>
<tr>
<td>Minerals</td>
<td>0.7g</td>
<td>1.0g</td>
<td>0.8 g</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>1.5-2.0 g</td>
<td>-</td>
<td>4.4 g</td>
</tr>
<tr>
<td>Vit A (β-carotene)</td>
<td>18000 µg</td>
<td>600 µg</td>
<td>174 µg</td>
</tr>
<tr>
<td>Vit E (α-tocopherol)</td>
<td>1 IU</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vit B1 (thiamin)</td>
<td>0.31 mg</td>
<td>0.1 mg</td>
<td>0.05 mg</td>
</tr>
<tr>
<td>Vit B2 (riboflavin)</td>
<td>0.35 mg</td>
<td>0.4 mg</td>
<td>0.19 mg</td>
</tr>
<tr>
<td>Vit B3 (niacin)</td>
<td>1.46 mg</td>
<td>0.1 mg</td>
<td>0.1 mg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>1 µg</td>
<td>70.3 µg</td>
<td>5.6 µg</td>
</tr>
<tr>
<td>Calcium</td>
<td>100 mg</td>
<td>60 mg</td>
<td>120 mg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>90 mg</td>
<td>220 mg</td>
<td>90 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>15 mg</td>
<td>2.1 mg</td>
<td>0.2 mg</td>
</tr>
<tr>
<td>Selenium</td>
<td>2 µg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Energy</td>
<td>36 Kcal</td>
<td>173 Kcal</td>
<td>67 Kcal</td>
</tr>
<tr>
<td>Phycocyanin (blue)</td>
<td>1500-2000 mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chlorophyll (green)</td>
<td>115 mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carotenoids (orange)</td>
<td>37 mg</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Source: - www.nbent.com and www.parrynutraceuticals.com

Spirulina is a rich source of vitamins especially vitamin B<sub>12</sub>, β-carotene (provitamin A), vitamin E (Belay, 1997). Spirulina is an adequate source of minerals like copper, magnesium, zinc, potassium, phosphorus, manganese, chromium, calcium and iron (Belay, 1997). Spirulina also contains 4-7 percent of lipids- essential fatty acids like gamalinolenic acid (GLA) which is required for arachidonic acid and prostaglandin synthesis (Othes and Pire, 2001). It is a host of other phytochemicals that have potential health benefits (Foster and Tyler, 1999; Belay, 2002).

Manoj et al., (1992) reported that the alcohol extract of Spirulina inhibited lipid peroxidation more significantly (65% inhibition) than the chemical antioxidants like α-tocopherol (35%),
BHA (45%), and β-carotene (48%). The water extract of Spirulina was also shown to have more antioxidant effect (76%) than gallic acid (54%) and chlorogenic acid (56%). An interesting aspect of their findings is that even the water extract had a significant antioxidant effect.

The in-vivo antioxidant capacity was evaluated in plasma and liver of animals receiving a daily dose of 5 mg for 2 and 7 weeks. Plasma antioxidant activity in brain homogenate incubated at 47°C showed that the antioxidant capacity of plasma was 97% and 71% for the experimental group and 74% and 54% for the control group after 2 and 7 months. The antioxidant effect was attributed to beta carotene, tocopherol, and phenolic compounds working individually or in synergy (Miranda et al., 1998).

Antioxidant effects of two fractions of a hot water extract of Spirulina were studied using three systems that generate superoxide, lipid, and hydroxyl radicals. Both fractions showed significant capacity to scavenge hydroxyl radicals (the most highly reactive oxygen radical) but no effect on superoxide radicals. One fraction had significant activity in scavenging lipid radicals at low concentrations. The antioxidant effect was attributed to beta carotene, tocopherol, and phenolic compounds working individually or in synergy acid metabolism (Belay, 2002).

2.12.3. Dosage of Spirulina for Supplementation

Daily intake of 3-5g of spirulina is found appreciable www.spirulina.com. The effect of spirulina at a dose of 15 mg/kg body yielded a higher level of significance than the doses of 5 and 10 mg/kg body weight (Layam and Chandra, 2006).

In a human clinical study involving 15 diabetics, a significant decrease in the fasting blood sugar level of patients was observed after 21 days of 2 g/day Spirulina supplementation. In a double-blind-crossover study versus placebo, Becker et al., (1986) have found that a supplementary diet of 2.8 g of Spirulina 3 times per day
over 4 weeks resulted in a statistically significant reduction of body weight in obese outpatients (Belay, 2002).

For adults of 18 years and older 1-1.4 grams of spirulina can be taken by mouth two to three times daily with meals in conditions like diabetes mellitus (type 2), high cholesterol, or oral leukoplakia (pre-cancerous mouth lesions). For weight loss, 200 milligrams of spirulina can be taken by mouth three times daily, before food. Two grams of spirulina has been used for nasal allergies. (http://www.nlm.nih.gov/druginfo/spirulina and www.naturalstandard.com).

Extraction of biomolecules from spirulina is widely done using different methods like diffusion, osmosis, solvent extraction procedures etc of which solvent extraction using ethanol has highest yield (10.9%) than other solvents (Herrero et al., 2004). Methods of extracting the intercellular components from the biomass are freezing and thawing of cells, homogenization using mortar and pestle, homogenization using a blender at high rpm, ultrasound assisted extraction; supercritical fluid extraction and accelerated solvent extraction were commonly employed for the extraction (Sarada et al., 1999). Common bioactives extracted from spirulina are gamalinolenic acid (GLA) (Sajilata et al., 2008), various antioxidants (Mendiola et al., 2007), vitamins, phytohormones, organic acids (Materasi et al., 1980) and unique polysaccharides (Schwartz and Shklar, 1990).

2.12.4. Health and Healing Effects

Khan et al., (2005) states that spirulina, possesses diverse biological activities and nutritional significance due to high concentration of natural nutrients, having bio-modulatory and immuno-modulatory functions, like increasing phagocytic activity of macrophages, stimulating the production of antibodies and cytokines, increase accumulation of NK cells into tissue and activation and mobilization of T and B cells. Ray et al., (2007) suggest that lipid peroxidation can be halted to a significant extent.
with the water extract of the algae. The anti-peroxidative effects of water extract of spirulina and its potential to reduce cisplatin-induced lipid peroxidation. The antioxidant effect of spirulina is attributed due to its various constituents working individually or in synergy.

Spirulina have also shown to perform regulatory role on lipid and carbohydrate metabolism by exhibiting glucose and lipid profile correcting activity in experimental animals and in diabetic patients. Preparations have been found to be active against several enveloped viruses including herpes virus, cytomegalovirus, influenza virus and HIV. They are capable to inhibit carcinogenesis due to anti-oxidant properties that protect tissues and also reduce toxicity of liver, kidney and testes (Khan et al., 2005).

Spirulina contains phycocyanin (7% dry weight basis) having antioxidant properties and have a direct effect on reactive oxygen species. It also contains an important enzyme superoxide dismutase (1700 units/g) that acts indirectly by slowing down the rate of oxygen radical generating reactions (Belay, 2002). It has been reported that spirulina has a protective role in cisplatin-induced nephrotoxicity in rats (Mohan et al., 2006). It was found that besides antioxidant effects spirulina had properties like immune-modulation effects (Hayashi et al., 1994), anticancer effects (Mathew et al., 1995).

2.13. FOOD SUPPLEMENTATION – RELATED STUDIES

According to the Dietary Supplement Health and Education Act (DSHEA) of 1994, a dietary supplement is a product that contains substances like vitamins, minerals, foods, botanicals, amino acids and is intended to supplement the usual intake of these substances. Dietary supplements are found in pill, tablet, capsule, powder or liquid form and are meant to be taken by mouth (Jegtvig, 2006). Supplementary Foods or Food Supplements are concentrates of important nutrients such as vitamins, minerals, polyunsaturated fatty acids, herbs and other food substances; which are used to remedy dietary deficiencies, and provide extra nutrients.
for those with special requirements caused by physiological conditions or a way of life to ensure an adequate intake of essential micro-nutrients. ([http://www.webhealthcentre.com/HealthyLiving/diet_nutrition](http://www.webhealthcentre.com/HealthyLiving/diet_nutrition))

The Natural Nutritional Foods Association estimated that in 2003 nutritional supplements amounted to a $19.8 billion market in the United States. The nutritional supplement industry provides a huge array of products for consumer needs (Layam and Chandra, 2006).

Nutritional status largely depends on the feeding habits of the community which are influenced by social customs, beliefs, superstitions, religion, cultural behavior, changes in the living environment, mass communication and the socio-economic status of the families. These socio-cultural factors play a vital role on the ecology of malnutrition over and above the nutritional and medical factors (Kumari and Devadas, 1990).

Anitha and Chandralekha, (2010) echoes that in this health conscious age, the blue-green algae, Spirulina is the nature’s most nutritious wholesome organic food with proven beneficial therapeutic properties. It is fast emerging as a whole answer to the varied demands due to its impressive nutrient composition which can be used for therapeutic uses. They also states that Spirulina has hypoglycemic and hypocholesterolemic effect which helps the diabetics to have control on blood glucose levels and also to deter the complications. Akbar et al., (2011) found that HbA1C levels were significantly reduced by antioxidant supplementation, suggesting that antioxidants may have some benefit in protecting against the complications of type 2 diabetes.

Different studies on the effect of spirulina and other antioxidant rich food supplementation on Diabetic subjects are abstracted below:

(1) According to Takai et al., (1991), a water-soluble fraction of Spirulina was found effective in lowering the serum glucose level
at fasting while the water-insoluble fraction suppressed glucose level at glucose loading.

(2) The possible mechanism by which spirulina brings about its anti-hyperglycemic action may be through potentiation of the pancreatic secretion of insulin from islet β-cell or due to enhanced transport of blood glucose to the peripheral tissue. This was clearly demonstrated by the increased levels of insulin and C-peptide in diabetic rats treated with spirulina (Layam and Chandra, 2006).

(3) In a human clinical study involving 15 diabetics, a significant decrease in the fasting blood sugar level of patients was observed after 21 days of 2 g/day spirulina supplementation (Hosoyamada et al., 1991; deCaire et al., 1995).

(4) Becker et al., (1986) have found that a supplementary diet of 2.8 g of Spirulina 3 times per day over 4 weeks resulted in a statistically significant reduction of body weight in obese outpatients.

(5) Likidlilid et al., (2007) aimed to compare the glutathione (GSH) level and glutathione peroxidase (GPx) activity in type 1 DM and a normal healthy group. GSH level and GPx activity were determined in red cells of 20 subjects of type 1 DM containing fasting plasma glucose (FPG) > or = 140 mg/dL. Twenty healthy normal subjects with normal plasma glucose level (FPG < or = 110 mg/dL) and matched for gender and age served as the control group. It was found that the level of red cell GSH was significantly lower in type 1 DM (p = 0.011) but red cell GPx activity was significantly increased (p = 0.003) when compared to age-matched normal control. The decrement of red cell GSH may be due to the higher rate of consumption of GSH, increasing GPx activity or a reduction of pentose phosphate pathway, stimulated by insulin, resulting in lowered GSH recycle. The correlation between FPG and GSH in type I diabetic patients compared with healthy normal subjects was also observed and it was found that there was a negative correlation, but not found between FPG and GPx activity. Likidlilid et al., (2007) suggested that type 1 DM patients were susceptible to oxidative stress and higher blood glucose level had an
association with free-radical-mediated lipid peroxidation. Therefore, any means that can reduce oxidative stress may be beneficial for slow progression of cardiovascular complication in type 1 diabetic patients.

(6) Medina et al., (2007) found that an increase in oxidizing response above a certain threshold produces oxidative stress that is associated with complications in diabetes, in the absence of a concomitant rise in antioxidant /reducing response. Plasma from healthy subjects (n = 15) showed significantly higher antioxidant status (p < 0.05) compared with plasma from diabetic patients (n = 27). The antioxidant status of plasma, reflect an antioxidant response since ROS generation was rapidly down-regulated by the presence of plasma which is 3.3-fold in diabetic patients and 5.8-fold in healthy subjects. It confirms the lower antioxidant activity of plasma from diabetic patients.

(7) Torres-Duran et al., (2007) conducted a study to evaluate the effects of Spirulina maxima orally supplied (4.5 g/day, for 6 weeks) to a sample of 36 subjects (16 men and 20 women, with ages between 18–65 years) on serum lipids, glucose, aminotransferases and on blood pressure. The volunteers did not modify their dietary habits or lifestyle during the whole experimental period. From each subject, a sample of blood was drawn in fasting state of 12 hours to determine the plasma concentrations of glucose, triacylglycerols (TAG), total cholesterol (TC), high density lipoprotein (HDL-C) and aspartate aminotransferase (AST). Anthropometric measurements including systolic (SYST-P) and diastolic (DIAST-P) blood pressure, height, weight and Body Mass Index (BMI) were also recorded. Comparing initial and final data, the results showed significant differences in TAG, TC, and HDL-C, were observed: The Spirulina maxima showed a hypolipemic effect, especially on the TAG and the LDL-C concentrations but indirectly on TC and HDL-C values. It also reduces systolic and diastolic blood pressure.
Montonen et al., (2004) reported a study on the dietary antioxidant intake and risk of type 2 diabetes. A cohort of 2,285 men and 2019 women of 40-69 years of age were studied. Food consumption was estimated use in a dietary history interview. The result shows that vitamin E intake was significantly associated with a reduced risk of type 2 diabetics. In takes of alpha tocopherol, gamma tocopherol, delta tocopherol and Beta tocotrienol, were inversely related to a risk of type 2 diabetes. Among single carotenoids, beta cryptoxanthin intake was significantly associated with a reduction to risk of type 2 diabetes. No association was evident between intake of vitamin C and type 2 diabetes risk. The study supports the hypothesis that development of type 2 diabetes may be reduced by the intake of antioxidants in the diet.

Dosoo et al., (2001) studied on total antioxidant status in non insulin dependent diabetes mellitus patients. The study was conducted in Ghana including 35 non insulin dependent diabetes Mellitus (NIDDM) patients aged 40-65. Patients were on diet and oral hypoglycemic drug. Similar measurements were carried out in 34 apparently healthy individuals within the same range. Comparing the two groups, total antioxidants status was significantly reduced in the NIDDM patients. An inverse correlation between fasting plasma glucose and total antioxidants status suggest the existence of lower antioxidants defense in uncontrolled NIDDM.

Svilaas et al., (2004) reported a study on the intake of antioxidants in coffee, wine, and vegetables and are correlated with plasma carotenoids in humans. A seven-day weighed dietary records in a group of 61 adults with corresponding plasma samples and used data from nationwide survey of 2672 Norwegian adults. The objective of the study was to determine the contribution of various food groups to total antioxidants intake and to assess the correlations of the total antioxidants intake from various food groups with plasma antioxidants. The study shows light to the fact that the intake of total antioxidant was significantly correlated with plasma lutein, Zeaxanthin and Lycopene. Among
individual food groups, coffee, wine and vegetables were significantly correlated with dietary Zeaxanthin, beta carotene and alpha carotene.

(11) Giammarioli et al., (2004) reported a study on the effect of high intake of fruits and vegetables on redox status in type 2 onset diabetes the purpose of the study was both to obtain preliminary data on the effect of a diet high in fruit and vegetables on metabolic control and the oxidative status of patients with type 2 onset diabetes, and to identify the most useful biochemical parameter for future research. At the beginning of the study all the subject were ask to follow their usual diet and to keep a seven day food diary. Diabetic patients then receive a dietary treatment design to ensure a daily intake of 700-1000 g of fruits and vegetables; no dietary advice was given to controls. Dietary antioxidants, redox status markers and parameter of metabolic control were measured in plasma and erythrocytes before and after the diet. Before the diet diabetic patients had lower levels of ascorbic acid, beta carotene and alpha tocopherol ratio than controls. After the diet these parameters increased and there was also a reduction in total antioxidant capacity, uric acid etc and a rise in reduce glutathione accompanied by a reduction in body mass index and cholesterol. It was concluded that a high consumption of fruit and vegetables by diabetic patients not receiving pharmacological treatment seems to produce an improvement in some redox status parameters.

(12) Sies et al., (2005) studied on nutritional, dietary and post pyramidal oxidative stress. The study shows that low intake or impaired availability of dietary antioxidants including vitamin E and C, carotenoids, polyphenols and other micronutrients (e.g. Selenium), weakens the antioxidants network. Postprandial oxidative stress as a sub form of nutritional oxidative stress ensures stained postprandial hyperlipidemia and/or hyperglycemia and is associated with a higher risk for atherosclerosis, diabetes and obesity. Postprandial oxidative stress is attenuated when dietary antioxidants are supplied together with a mean rich in oxidised or
oxidisable lipids. Injection for dietary polyphenols from wine, cocoa, or tea improves endothelial dysfunction and lowers the susceptibility of LDL lipids to oxidation. Polyphenols affect endothelial function not solely as antioxidant but also as a modulatory signaling molecule.

(13) Osawa and Joji, (2005) studied on protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. He suggested that hyperglycemia caused the auto oxidation of glucose, glycation of protein and the activation of polyol metabolism. These changes accelerate generation of reactive oxygen species (ROS) and increase in oxidative chemical modification of lipids, DNA and protein in various tissues. Oxidative stress may play an important role in the development of complications in diabetes such as cataract, nephropathy and neuropathy. Glycation reaction especially maillard reaction occur in vivo as well as in vitro and are associated with chronic complications of diabetes mellitus and aging and age related diseases by increases in oxidative chemical modification formed by DNA and proteins.

(14) Rube and McDonald, (2001) reported a study on the use of antioxidant nutrients in the prevention and treatment of type 2 diabetes. They suggested that an alternate approach to the control of type 2 diabetes is to arrest the progress of pathology until a cure has been found. For that dietary antioxidants may be of value. Vitamin E and lipoic acid supplements lessen the impact of oxidative damage caused by dysregulations of glucose metabolism.

(15) Niedowicz and David, (2005) studied the role of oxidative stress in diabetic complications. The study explores the production of reactive oxygen species and the propagation and consequences of oxidative stress in diabetes. He suggested that Reactive oxygen species are produced by oxidative phosphorylation, nicotinamide adenine dinucleotide phosphate oxidase (NADPH), Xanthine oxidase, the uncoupling of lipoxygenases, cytochrome mono-oxygenase and glucose auto oxidation. Once formed,
ROS deplete antioxidant defenses, rendering the affected cells and tissues more susceptible to oxidative damage. Reactive oxygen species are also important as second messengers in the regulation of intracellular signaling pathways and gene expression.

(16) A study on free radicals, antioxidants and nutrition by Fang et al., (2002) reveals that dietary antioxidants are useful radio protectors and play an important role in preventing many human diseases like diabetes. The study pointed out that production of reactive oxygen species result in damage to biomolecules and are produced as a result of aerobic metabolism. Antioxidants (e.g. Selenium, Zinc, Vitamin E, Vitamin C, Glutathione, Arginine, Citrulline. Taurine, Creatinine and Polyphenols) and antioxidant enzymes (e.g. Superoxidedismutase, Catalase, Glutathione reductase and peroxidase) exert synergistic actions in scavenging free radicals.

(17) Padma and Anitha, (2005) studied effect of heat on the antioxidant status of selected green leafy vegetables. The study points out that diets rich in fruits and vegetables that are rich sources of antioxidant vitamins like beta carotene, ascorbic acid and tocopherols, lower the incidence of number of diseases. The study was conducted on fresh leafy vegetables and subjected to boiling. They concluded that boiling resulted in a drastic loss of catalase and peroxidase activity.

2.14. CONCLUSION

In the absence of effective interventions, diabetes will continue to increase its frequency worldwide. In a country like India prevention of diabetes and its consequences is not only a major challenge for future but essential, if health for all is to be an attainable target. Though Genetic Research can once reverse the epidemic diabetes, the major query is when it will be available to even the poorest of the poor in the world.

The right diet combined with other suitable measures is
a powerful tool which could keep this silent malaise at bay. Dietary antioxidants have a protective effect against the development of diabetes by inhibiting per-oxidation chain reactions. It seems plausible that, sufficient intake of antioxidants plays an important role in protection against diabetes.

Various research activities are going on in different parts of the world for extracting therapeutic potentials from spirulina. India being the diabetic capital, there is considerable scope for research in spirulina processing for developing various types of food products which can be used in our day today life. Food scientists and technologists in India should focus on developing new ready to eat products containing active health ingredients from spirulina especially with the Indian taste and food culture. Future investigation is needed to remove the off flavor and taste without change in nutritive value so that it does not hinder consumer acceptance.

The major gap areas in the field of antioxidants and diabetes mellitus are:

Researchers have already revealed the rates of prevalence of type II diabetes mellitus in several sectors, but such information on the socio-economic, dietary pattern, life style pattern, health and clinical status of type I diabetic patients has not been unearthed among the residence of Thiruvanathapuram district.

To the full of our knowledge the antioxidant status of the people of Kerala have not been studied so far. Comparison on the antioxidant status of non-diabetic and diabetic subjects especially the type I and type II subjects is essential to the future policy makers for the accurate estimation of these burdens, their risk factors, and time trends and tackles the present situation.

The primary need of a diabetic patient is to attain and sustain normo-glycemia. Management and treatment of diabetes is difficult due to lack of awareness, lack of education
and health care facilities. Proper dietary management is essential from early stage of human life to sustain health and wellbeing. Hence educational packages are to be developed that reaches every nook and corner of the State.

Although there are a number of drugs available on the market for diabetes mellitus, which are of high cost, its long time use may cause side effects. To develop newer, cheaper, and safer natural supplements which can effectively normalize the metabolic derangement underlying the onset of clinical diabetes is essential. Products developed out of Spirulina may increase consumer acceptance.