Chapter-1
Introduction
Introduction

Chapter-1

The list of complex disorders is long and includes world’s leading causes of death like cancer, cardiovascular diseases and diabetes. The World Health Organization world health statistics report shows that in the coming 25 years the proportion of deaths due to non-communicable diseases will raise significantly (WHO, 2008). Diabetes which was at 12\textsuperscript{th} position by accounting for 1.9% deaths will shift to 7\textsuperscript{th} position by contributing 3.3% of the deaths.

Diabetes mellitus is a group of diseases characterized by an elevated blood glucose level (hyperglycemia) resulting from defects in insulin secretion, in insulin action, or both. It is not a pathogenic entity but a group of etiologically different metabolic defects. Common symptoms of diabetes are lethargy from marked hyperglycemia, polyuria, polydipsia, weight loss, blurred vision and susceptibility to certain infections. Severe hyperglycemia may lead to hyperosmolar syndrome and insulin deficiency to life-threatening keto-acidosis. Chronic hyperglycemia causes long-term damage, dysfunction and failures of various cells, tissues and organs.

1.1. Classification of diabetes mellitus

There was several classification systems established for diabetes mellitus by the WHO Expert Committee on Diabetes (WHO 1980, 1985). The current WHO classification system as given below has been established in co-operation with the National Diabetes Data Group (USA) and is mainly based on the etiology of diabetes mellitus (Reinauer, 2002).
Chapter – 1

Introduction

Type 1 diabetes

Type 2 diabetes

Gestational diabetes mellitus (GDM)

Type 1 diabetes

It was formerly known as insulin dependent diabetes mellitus (IDDM) or juvenile diabetes. Type 1 diabetes is characterized by cellular-mediated autoimmune destruction of islet β-cells. Age of onset is predominantly in childhood and adolescence. This form of diabetes is strongly inherited, has permanent insulinopenia and is prone to keto-acidosis without antibodies to β-cells. Symptoms include hyperglycemia, ketonuria, low or undetectable serum insulin and c-peptide levels and auto-antibodies against components of the islet β-cells. Markers useful in the diagnosis of Type 1 diabetes are listed below.

- Islet cell antibodies (ICAs)
- Auto-antibodies to insulin (IAAs)
- Auto-antibodies to glutamic acid decarboxylase (GAD65)
- Auto-antibodies to tyrosine phosphatases IA-2 and IA-2β

Type 2 diabetes

It was previously known as maturity onset diabetes and non-insulin dependent diabetes mellitus (NIDDM). Type 2 diabetes develops due to insulin insensitivity combined with a failure of insulin secretion, causing high blood glucose level. This is the most common form of diabetes. Observed symptoms in type 2 diabetes are hyperglycemia, hyperlipidaemia, high serum insulin level, defective insulin secretion and insulin resistance.
Chapter – 1  

Introduction

Gestational diabetes mellitus

Any degree of clinical glucose intolerance with onset or first recognition during pregnancy is considered as Gestational Diabetes Mellitus (GDM). The following problems may develop with GDM.

- Altered duration of pregnancy
- Placental failure
- Hypertension / pre-eclampsia
- High birth weight of the newborn

1.2. Global epidemiology of diabetes

Diabetes is one of the major complex disorders in humans. In the spectrum of diabetic disorders type 2 diabetes is the most common form of diabetes, accounting for more than 90% of world’s diabetic population (WHO, 1999). International diabetic federation estimates that 246.1 million individuals within the age group of 20-79 years (5.9% of the world population in the age group) are suffering from diabetes (IDF, 2006). Western Pacific region has highest number of diabetic individuals (64 million), followed by Europe (53.2 million) and South East Asia (46.5 million).

When it comes to the prevalence, Eastern Mediterranean and Middle East was having highest occurrence of diabetes (9.2%), followed by North America (8.4%) (Table 1.1). Between 1995 and 2025, about 35% increase in the world-wide prevalence of diabetes has been predicted and the rising number of individuals with the disease will occur mainly in populations of the developing countries, leading to more than 300 million individuals with diabetes globally by 2025 (Reinauer, 2002). More than 80% of the 246 million individuals with diabetes reside in the low and
middle income countries, providing substantial evidence to suggest that diabetes is no longer a disease of the developed countries.

During the year 2007, an estimated 40.9 million diabetic individuals within the age group of 20-79 years were residing in India which makes it top the list with highest number of diabetic individuals in the world, followed by China (39.8 million). Though India has highest number of diabetics, Nauru was having the highest prevalence of diabetes (30.7%) in the world, followed by United Arab Emirates (19.5%) (IDF, 2006). From 1995 to 2025, increase in diabetes prevalence was expected to be 42% (from 51 to 72 million) in the developed countries and 170% (from 84 to 228 million) in the developing countries. According to King et al. (1998) the countries with the largest number of diabetic individuals were and will be India and China. These numbers might be under estimates because, due to its late age of onset, individuals may live for years with type 2 diabetes by the time they get diagnosed.

1.3. The Indian scenario

As there were no standard criteria for conducting surveys to diagnose diabetes, in the earlier days most of the studies were based on hospital records and glycosuria was used as the diagnostic criteria. In India the first documented study was conducted in Calcutta, Bengal, where 1% of individuals were found to be diabetic out of 96,300 studied medical records (Chakravarthy, 1938). In Mumbai where eight years case records were analyzed, the prevalence of diabetes was 0.7% (Patel and Dhirawani, 1958). The first hospital based study was conducted on 63,356 individuals in South India which reported a prevalence of 2.5% (Vaishnava et al., 1964). High prevalence (8.7%) was reported at Trivandrum, Kerala; the study was based on hospital records (Pai et al., 1966). House to house surveys at Chandigarh, Pondicherry and Varanasi
reported prevalence rates of 2.9, 0.7% and 2.7%, respectively (Berry et al., 1966; Data et al., 1966; Gour, 1966).

Population based surveys in different Indian cities and nearby rural areas reported prevalence of diabetes ranging from 1.2 to 2.5% (Tripathy et al., 1971; Vigg et al., 1972; Ahuja et al., 1972; Parameshwara, 1973; Rao et al., 1972). Indian Council of Medical Research (ICMR) conducted first multicentric study in which six different cities of India were selected and 34,000 individuals were screened for diabetes; capillary blood glucose level >170mg/dl was defined as diabetic phenotype. According to the study results, prevalence of diabetes in urban and rural areas was 3.0% and 1.3%, respectively (Gupta et al., 1978). Considering these studies it is evident that till 1970s the prevalence of diabetes was not more than 3.0% in urban and rural areas of India, the only exception being Trivandrum.

There was a steady increase in the prevalence of diabetes in India in the next decades; a little high prevalence of the disease was reported in Tenali (4.7%), a town in Andhra Pradesh (Murthy et al., 1984). Prevalence in Bhadlan, a rural area in Haryana, was 3.8% which was relatively higher than earlier surveys (Patel, 1986). The Daryaganj diabetes survey observed 3.1% prevalence rate in an affluent neighbourhood of Delhi (Verma et al., 1986). A study by Ramachandran et al. (1988) in Kudremukh, South India reported a prevalence of 5.0%. The prevalence of diabetes in Eluru survey of rural Andhra Pradesh was 1.5%; however in individuals aged above 40 yr the prevalence of known diabetes was 6.1% which was unexpectedly higher for a rural area (Rao et al., 1989).

Ahuja (1991) carried out a multicentric study in rural areas near Ahmedabad, Calcutta, Delhi and Thiruvananthapuram and found the
prevalence rates as 3.9%, 0.8%, 1.5% and 1.3%, respectively and remote high altitude area of Kalpa, Himachal Pradesh, had a prevalence of 0.4%. The same study revealed that the prevalence was 4.1% in an industrial neighbourhood of Delhi. Wander et al. (1994) reported 5% prevalence of diabetes by considering random venous blood glucose level >180mg/dl or history of diabetes in a rural population of Ludhiana, Punjab. Another study was reported from a rural area of North Arcot district, Tamil Nadu in which 467 subjects aged 40 yr or above were selected and given 75 g glucose orally and after two hours the capillary blood glucose levels were determined. Prevalence of impaired glucose tolerance (IGT) (2h value ≥ 7.8 mmol/L and ≤ 11.1 mmol/L) was 6.6% and prevalence of non-insulin dependent diabetes mellitus (2h value ≥ 11.1 mmol/L) was 4.9% (Patandin et al., 1994). Most of these studies indicated that the prevalence of diabetes increased in both urban and rural areas of India after 1970s.

Further evidence for increasing trend in diabetes incidence came from a study conducted in Chennai during 1988-1995 in which prevalence of diabetes and IGT was found to be 8.2% and 8.7%, respectively (Ramachandran et al., 1992). After five years, follow up study reported that the prevalence of type 2 diabetes and IGT rose to 11.6% and 9.1%, respectively (Ramachandran et al., 1997).

In Kashmir valley, 1.89% of population was having known diabetes, 4.25% undiagnosed diabetes and 8.09% had IGT (Zargar et al., 2000). Iyer et al. (2001) reported that the prevalence of diabetes as per WHO (1996) criteria was 4.61% in an urban population of Maharashtra; but as per American Diabetes Association (ADA) criteria it was 7.5%. In the Chennai Urban Population Study (CUPS) involving two areas in Chennai representing the middle and lower socio-economic groups, the overall prevalence of type 2 diabetes was 12% in subjects aged above 20 yr
Chapter – 1

Introduction

(Mohan et al., 2003). The middle income group had significantly higher prevalence of type 2 diabetes compared to the low income group, age standardized prevalence rates being 12.4% and 6.4%, respectively; the prevalence of IGT was 5.9% (age standardized prevalence 5.0%). The prevalence of glucose intolerance was notably high (55%) in subjects who had diabetic parents; subjects with less physical activity also showed considerable prevalence (23.2%).

The National Urban Diabetes Survey (NUDS) was a population based study covering six major cities of India in which 11,216 subjects aged over 20 yr from all socio-economic strata were selected. The diabetes was diagnosed as per WHO (2006) criterion after an OGT test using capillary blood. The study showed that age standardized prevalence of type 2 diabetes was 12.1% and highest prevalence was reported in Hyderabad (16.6%), followed by Chennai (13.5%), Bengaluru (12.4%), Kolkata (11.7%), New Delhi (11.6%) and Mumbai (9.3%) (Ramachandran et al., 2001).

Similar to CUPS, The Chennai Urban Rural Epidemiology Study (CURES) showed a prevalence of 15.5% (age standardized 14.3%) in Chennai, Tamil Nadu. From this study it was clear that the prevalence of diabetes has increased by 39.8% (from 8.3 to 11.6%) during 1989-1995; 16.3% (11.6-13.5%) between 1995 and 2000 and 6.0% (13.5-14.3%) between 2000 and 2004. By 2006, within a span of 14 years, the prevalence of diabetes increased by 72.3%, (p < 0.0001) in Chennai (Mohan et al., 2006a).

The Prevalence of Diabetes in India Study (PODIS) was another diabetic study carried out in India in which 49 urban and 59 rural areas from different parts of the country were selected. Prevalence of diabetes was found to be 5.9% and 2.7%, respectively, in the urban and rural
Chapter – 1  

Introduction

populations, whereas IGT rates were 6.3 and 3.7%, respectively. Prevalence of diabetes and IGT was significantly more in urban population (p < 0.001 for both) (Sadikot et al., 2004). Amrita Diabetes and Endocrine Population Survey (ADEPS) was a community based cross sectional survey conducted in urban areas of Ernakulam district of Kerala to assess the prevalence of undetected diabetes mellitus and IGT. In this study adults between 18 and 80 yr of age were selected in house to house surveys, followed by health checkup and biochemical evaluations. Diabetes and IGT were diagnosed as per WHO (1996) criteria. The prevalence of known diabetes mellitus in the survey participants was 9.0% while that of newly diagnosed diabetes was 10.5% and that of IGT was 4.1% (Menon et al., 2006).

Studies conducted in India in the last decade highlighted that not only the prevalence of type 2 diabetes has risen, but also that it is increasing rapidly in the urban population (Mohan et al., 2001b; Kutty et al., 2000; Misra et al., 2001a; Verma and Madhu, 2000; Ramachandran et al., 2008). Statistically significant increase in the trend of diabetes (p < 0.001) was observed in three studies conducted in Chennai during 1989 (Ramachandran et al., 1992), 1995 (Ramachandran et al., 1997a) and 2000 (Ramachandran et al., 2001). An urban rural difference in the prevalence rate indicates the rapid economic development and that urbanization had a significant role in increasing prevalence of diabetes. Epidemiological studies conducted on migrant Asian Indians showed increased risk of type 2 diabetes among Indians than native populations (Abate and Chandalia, 2001; Abate et al., 2004; Abate and Chandalia, 2007).

Indians have a younger age of onset of diabetes compared to other ethnic groups (Ramachandran et al., 2008). An increase in the prevalence of type 2 diabetes in the younger age group has been noted from the
epidemiological studies. NUDS study showed that the prevalence of diabetes in subjects aged below 30 yr was 5.4% (Ramachandran et al., 2001). When compared with the NUDS study, the CURES investigators demonstrated a temporal shift in the age of onset of diabetes to a younger group (Ramachandran et al., 2001; Mohan et al., 2006a). Hence it is clear that type 2 diabetes has become prevalent even among younger age groups and this could have long lasting effects on the health of the nation and its economy.

Some studies have reported that the prevalence of diabetes was higher in females compared to males (Kutty et al., 1999; Ramachandran et al., 1992). The population based multi centric studies like NUDS and PODIS reported similar prevalence in males and females (Ramachandran et al., 2001; Sadikot et al., 2004). Impaired glucose tolerance and impaired fasting glucose (IFG) collectively called as prediabetic states, have a high risk of conversion to diabetes. Studies have shown that these prediabetic states are also having high risk for cardiovascular diseases (Novoa et al., 2005).

The prevalence of diabetes related metabolic abnormalities like obesity and cardiovascular risk factors were also markedly higher in the middle income group. A study from New Delhi reported that even the slum dwellers had high prevalence of obesity, glucose intolerance and dyslipidemia (Misra et al., 2001b). This suggests diabetes is no longer a disease of the affluent or rich; it is becoming a problem even among the middle income and poor sections of the society. Studies have shown that the poor diabetic subjects were more prone to complications as they have little access to quality health care (Mohan et al., 2007). This presents an alarming picture, as the poor would find it more difficult to cope with the diabetes epidemic.
Chapter – 1

Introduction

There are few studies from India which investigated the microvascular complications of diabetes. Rema et al. (1996) observed 34.1% prevalence of diabetic retinopathy (DR) in a clinic based study conducted in a diabetic centre in Chennai. During 1996-1997 a study conducted on the urban South Indian population revealed that the prevalence of DR was 22.6% (Dandona et al., 1999). Using a cluster sampling technique, Narendran et al. (2002) selected subjects aged 50 yr and older from Palakkad district of Kerala state and in 5,212 subjects examined, a high prevalence of retinopathy (26.8%) was found in the diabetics.

The CURES eye study was another large population based study which used four-field stereo colour photography to check the prevalence of DR. The overall prevalence of DR in the population was 17.6%, which included 20.8% known diabetic subjects and 5.1% subjects who were newly diagnosed with diabetes. The prevalence of DR was significantly higher in men than women (21.3% and 14.6%, respectively; p < 0.0001) (Rema et al., 2005). This prevalence was low compared to the reports from Western countries (Kohner et al., 1998).

A few studies have looked at the prevalence of diabetic nephropathy in India and most of these were clinic based reports. John et al. (1991) in a study from Vellore, Tamil Nadu, found that the prevalence of microalbuminuria and diabetic nephropathy was 19.7% and 8.9%, respectively. Elevated blood sugar and blood pressure were observed in patients with micro- and macro-albuminuria; vascular complications were also found to be higher in the macro-albuminuric group (p < 0.01). Diabetic retinopathy tends to occur more frequently in micro-albuminurics as the prevalence rate was found to be 26.6% in a study from North India (Gupta et al., 1991).

The prevalence of neuropathy in two separate clinic based studies from Chennai was found to be 27.5% by Ashok et al. (2002) and 19.1% by
Ramachandran et al. (1999). A recent study reported high prevalence of neuropathy (29%) in newly diagnosed diabetic subjects (Dutta et al., 2005). Population based data from CURES showed that the prevalence of neuropathy in urban population was 26.1% (Mohan et al., 2008). Foot ulcers were found to be more common among diabetic subjects in the rural areas compared to their urban counterparts and the rates of amputations were higher in rural areas compared to urban areas (Viswanathan et al., 2006).

The results from the CUPS study have provided population based data on the prevalence of macro-vascular complications and mortality in relation to diabetes in India. The prevalence of coronary artery disease was 21.4% among diabetic subjects compared to 9.1% in subjects with normal glucose tolerance (Mohan et al., 2001a). The prevalence of peripheral vascular disease (PVD) was higher in type 2 diabetic subjects compared to non-diabetic subjects (6.3% and 2.7%, respectively, p<0.001) (Premalatha et al., 2000).

The CUPS also provided some evidence on the effect of type 2 diabetes on mortality rates in a population. The overall mortality rates were nearly three-fold higher in diabetic subjects compared to non-diabetic subjects (18.9 and 5.3, respectively per 1000 person-years). The hazards ratio (HR) for all-cause mortality for diabetes was found to be 3.6 [95% CI 2.02-6.53, p<0.001] (Mohan et al., 2006b).

Several clinic based studies from India have looked at the mortality trends in diabetes. Coronary artery disease (CAD) appears to be leading cause of death in majority of these studies (Das et al., 1991). However, Bhansali et al. (2003) and Zargar et al. (1999) reported that infections were the leading cause of mortality in diabetic subjects.
1.4. *Type 2 diabetes risk factors*

Considering the high prevalence rate of type 2 diabetes in India in recent years, it is important to identify the risk factors which contribute for development of the disease. Since type 2 diabetes is a multifactorial disorder, the associated risk factors were classified into environmental and genetic risk factors; environmental risk factors include age, lifestyle and diet, among others.

The prevalence and incidence of type 2 diabetes increases dramatically as a function of age (Ramachandran et al., 2001). In developing countries, the majority of diabetes patients were found to be in the age range of 45-64 yr, whereas in the developed countries they were >65 yr (Harris et al., 1998). Younger age at onset of diabetes had been noted in the Asian Indians in studies conducted on migrants (Ramaiya et al., 1990b; Ramachandran et al., 2001). Socio-economic environment influences, occupation, lifestyle, and nutrition of different social classes influence the prevalence and profile of glucose intolerance and diabetic complications.

Prevalence of diabetes was found to be lower in the low socio-economic group living in urban areas compared with the high-income group (12.6% and 24.6%, respectively in subjects ≥ 40 yr) (Ramachandran et al., 2002b). This was probably related to the physical activity of the low income group (LIG), as most of them were involved in moderate to strenuous physical activity at work. Prevalence of diabetes and impaired glucose tolerance were significantly lower in the LIG than in the high income group (HIG). The finding of lower prevalence of diabetes in the socially deprived urban Indians was in contrast to the positive association of diabetes and social deprivation in western countries (Meadows, 1995; Unwin et al., 1996; Evans et al., 2000; Kelly et al., 1993). Unfortunately the poor diabetic subjects delay taking treatment
leading to increased risk of complications (Ramachandran et al., 2002b). Moreover availability of energy saving methods of transport and labour has resulted in severely reduced physical activity.

A recent population study in Chennai, Tamil Nadu showed that the total activity level considering the activity at work and during leisure time was very less, particularly in women. The activity score was inversely related to the affluence or wealth score, and low activity score showed adverse effects on glucose intolerance. The affluence was calculated using a wealth score system and a significant correlation was seen between increasing wealth score and decreasing total activity (Ramachandran et al., 2002c). Sedentary life style was one of the significant factors associated with diabetes in this population (Pocock et al., 1987; Ramachandran et al., 2002a). Along with these environmental risk factors, several occupations and occupational exposures were identified which might have contributed for disease prevalence. Regarding environmental contaminants as etiologic agents for diabetes, data on arsenic were suggestive but inconclusive (Ramachandran et al., 2002a).

According to WHO (Reinauer et al., 2002) the risk of developing type 2 diabetes increases with the following.

1. Membership of some ethnic groups.
2. Family history of diabetes, in particular parents or sibling of the proband being diabetic.
3. Obesity (≥ 20% over ideal body weight or BMI ≥ 25).
4. Previously identified impaired fasting glucose or impaired glucose tolerance.
5. Hypertension (≥ 140/90 mmHg) in adults.
6. HDL cholesterol level <1.0 mmol/l (<0.38 g/l) and/or a triglyceride level ≥ 3 mmol/l (≥2.0 g/l).
7. History of gestational diabetes mellitus (GDM) or delivery of babies >4-5 kg.

Prevalence of diabetes varies from population to population implying that certain populations in the world are at higher risk of developing it than the others. For example, the Pima Indians showed highest prevalence of the disease (Reinauer et al., 2002) and the Asian Indians showed increased susceptibility to diabetes (Meadows, 1995), due to higher percentage body fat, more centrally obese distribution (McKeigue et al., 1989) and more insulin resistance than Europeans (Ramachandran et al., 1997b).

To explain the population specific risk for type 2 diabetes, Neel (1962) proposed the thrifty genotype concept, according to which the genes which had selective advantage in the evolutionary period are now working as causative factors in the development of disease due to change in the environment and lifestyle of the population (Knowler et al., 1990). The genetic component of an individual contributes for the factors like BMI, hypertension and lipid levels, which are said to increase the risk of diabetes development (Iwasaki et al., 2003; Ortega-Alonso et al., 2009; Sonnenberg et al., 2004; York, 1975; Fuhrmann, 1974). The effect of BMI on the age adjusted prevalence of type 2 diabetes was modified by ethnicity with considerably lower thresholds in Indian subjects compared to those from Europe (Neel, 1962).

It was also showed that the concordance rates for diabetes in monozygotic twins, which ranged from 60-90%, were significantly higher than those for dizygotic twins (Nakagami et al., 2003). A study by Kumar et al. (2005) in North India reported that insulin resistance was observed
in high frequency in the first degree relatives (FDRs) of type 2 diabetes mellitus patients and this increases along with the progression of the disease, being highest in individuals with FDRs having type 2 diabetes mellitus. In addition to diabetes, the condition of impaired glucose tolerance (IGT) also constitutes a major health problem. IGT is an intermediate condition between normality and diabetes. Individuals with IGT are at high risk of progressing to type 2 diabetes, although such progression is not inevitable. The prevalence of IGT is more than twice that of diabetes in the African and South-East Asian regions.

1.5. Diabetes pathophysiology

The pathophysiology of type 2 diabetes mellitus is characterized by peripheral insulin resistance, impaired regulation of hepatic glucose production, and declining β-cell function eventually leading to its failure. The primary events in diabetes development are believed to be an initial deficit in insulin secretion, although in many patients relative insulin deficiency in association with peripheral insulin resistance has been observed (Reaven, 1988). β-cell dysfunction is initially characterized by an impairment in the first phase of insulin secretion during glucose stimulation and may antedate the onset of glucose intolerance in type 2 diabetes (WHO, 1999).

Transmembranous transport of glucose and coupling of glucose to the glucose sensor mediate the initiation of insulin response. The glucose/glucose sensor complex then induces an increase in glucokinase by stabilizing the protein and impairing its degradation. The induction of glucokinase serves as the first step in linking intermediary metabolism with the insulin secretory apparatus. Glucose transport in β-cells of type 2 diabetes patients appears to be greatly reduced, thus shifting the control point for insulin secretion from glucokinase to the glucose transport system (Leahy, 1990; Porte, 1991).
Later in the course of disease, the second phase, release of newly synthesized insulin impairs an effect that can be reversed in part at least in some patients by restoring strict control of glycemia. This secondary phenomenon termed desensitization or β-cell glucotoxicity is the result of a paradoxical inhibitory effect of glucose upon insulin release and may be attributed to the accumulation of glycogen within the β-cell as a result of sustained hyperglycemia (Ward et al., 1986). Other proposed candidates were sorbitol accumulation in the β-cell or the nonenzymatic glycation of β-cell proteins. β-cell function in type 2 diabetes mellitus include defective glucose potentiation in response to non-glucose insulin secretagogues, asynchronous insulin release, and decreased conversion of proinsulin to insulin (Porte and Kahn, 1989; O’Rahilly et al., 1988).

Impairment in first phase insulin secretion may serve as a marker of risk for type 2 diabetes mellitus in family members of individuals with type 2 diabetes mellitus (Eriksson et al., 1989; Vaag et al., 1995; Warram et al., 1990; Beck-Nielsen and Groop, 1994) and may be seen in patients with prior gestational diabetes (Nicholls et al., 1995). In the great majority of patients with type 2 diabetes (~80%), the delay in immediate insulin response is accompanied by a secondary hypersecretory phase of insulin release as a result of either an inherited or acquired defect within the β-cell or a compensatory response to peripheral insulin resistance. Over a prolonged period of time perhaps years, insulin secretion gradually declines possibly as a result of intra-islet accumulation of glucose intermediary metabolites (Malaisse, 1996).

Emanating from the hyper-insulinism in type 2 diabetes, insulin resistance has been considered to play an integral role in the pathogenesis of the disease (Kruszynska and Olefsky, 1996). Lean type 2 diabetic patients over 65 yr were found to be as insulin sensitive as their age matched non diabetic controls (Arner et al., 1991). Moreover, in the
majority of type 2 diabetic patients who were insulin resistant, obesity was almost invariably present (Kissebah et al., 1989; Björntorp, 1988). As obesity or an increase in intra-abdominal adipose tissue was associated with insulin resistance in the absence of diabetes, it is believed by some researchers that insulin resistance in type 2 diabetes is entirely due to the coexistence of increased adiposity (Yalow and Berson, 1960). Additionally, insulin resistance is found in hypertension, hyperlipidemia, and ischemic heart disease, entities commonly found in association with diabetes (Ferrannini and Stern, 1995; Nabulsi et al., 1995; Reaven, 1988), raising the question as to whether insulin resistance results from different pathogenetic disease processes or is unique to the presence of type 2 diabetes (Charles et al., 1997; Fagan and Deedwania, 1998; Reaven, 1988).

Prospective studies have demonstrated the presence of either insulin deficiency or insulin resistance before the onset of type 2 diabetes (Ferrannini and Stern, 1995). Two studies have reported the presence of insulin resistance in non-diabetic relatives of diabetic patients at a time when their glucose tolerance was still normal (Gelding et al., 1995; Gulli et al., 1992). In addition, first degree relatives of patients with type 2 diabetes have been found to have impaired insulin action upon skeletal muscle glycogen synthesis due to both decreased stimulation of tyrosine kinase activity of the insulin receptor and reduced glycogen synthase activity (Vaag et al., 1992; Gulli et al., 1992).

Other studies in this high risk group have failed to demonstrate insulin resistance, and in the same group, impaired early phase insulin release and loss of normal oscillatory pattern of insulin release have been described (Pimenta et al., 1995; O’Rahilly et al., 1988). Based on these divergent studies, it is still impossible to dissociate insulin resistance
from insulin deficiency in the pathogenesis of type 2 diabetes. However, both entities unequivocally contribute to the fully established disease.

The ability of insulin to suppress hepatic glucose production both in the fasting state and postprandial was normal in first degree relatives of type 2 diabetic patients (Eriksson et al., 1989). It is the increase in the rate of postprandial glucose production that heralds the evolution of IGT (Gelding et al., 1995). Eventually, both fasting and postprandial glucose production increase as the disease progresses. Hepatic insulin resistance is characterized by a marked decrease in glucokinase activity and a catalytic increased conversion of substrates to glucose despite the presence of insulin (Gulli et al., 1992). Thus, the liver in type 2 diabetes is programmed to both overproduce and underuse glucose. The elevated free fatty acid levels found in type 2 diabetes may also play a role in increased hepatic glucose production (Charles et al., 1997). In addition, recent evidence suggests an important role for the kidney in glucose production via gluconeogenesis, which is unrestrained in the presence of type 2 diabetes (Stumvoll et al., 1997).

In obesity, experimental evidence has confirmed the presence of insulin resistance in adipose tissue, muscle (Rabinowitz et al., 1962) and liver (Felig et al., 1974). As a consequence, when obese subjects develop diabetes, there is a weakened inhibition of splanchnic glucose output with simultaneous insulin increase and elevated concentrations of free fatty acids (FFA) (Reaven, 1988). However, the insulin resistance that accompanies obesity is related to a decrease in total body glucose disposal and was thought to occur predominantly in the skeletal muscle (Caro et al., 1989). It has been clearly demonstrated that both an increase in fatness and a preferential upper body accumulation of fat are independently related to insulin resistance (Clausen et al., 1996).
Chapter – 1

Introduction

The molecular revolution brought to light many adipocyte-secreted factors, some of which are secreted into the bloodstream such as IL-6, resistin, adiponectin and leptin (Trayhurn and Beattie, 2001). Whereas others such as TNF-α exert their effects in an autocrine–paracrine fashion. Large fat cells, evidence of increased energy influx, are likely to secrete an entirely different pattern of hormones than small adipocytes. For example, adiponectin concentrations are decreased in obesity and type II diabetes. Administration of adiponectin to rodents reduces triglyceride storage in the liver and muscle through AMP activated protein kinase α2 (AMPKα2) activation (Yamauchi et al., 2002).

1.6. Genetics of type 2 diabetes

The possibility of heredity playing part in the etiology of diabetes appears to have been originally suggested by Rondolet, a physician of Montpellier, France in the sixteenth century, before the idea was definitely formulated by Johann Peter Frank in the eighteenth century who also has the distinction of having differentiated diabetes mellitus from diabetes insipidus (Medvei, 1993). Most subsequent writers on diabetes mellitus accepted Frank’s view and published statistics showing the proportion of their cases in which family history of the disease had been traced (Cammidge, 1928).

Despite strenuous efforts over the past three decades to identify the genetic variants that contribute to individual differences in predisposition to type 2 diabetes, until recently the process was characterized by slow progress and limited success. However in recent years, identifying the genetic causes of type 2 diabetes got extensive support from the advances in molecular biology which facilitated researchers to identify various loci that are associated with the disease. The advent of genome wide association (GWA) analysis has transformed the potential for researchers to uncover variants influencing common complex phenotypes.
including type 2 diabetes, and has resulted in the identification of a growing number of trait susceptibility loci (Sladek et al., 2007; Saxena et al., 2007; Scott et al., 2007; Steinthorsdottir et al., 2007; Zeggini et al., 2007; Salonen et al., 2007).

Until 2006, main approaches used to track down common variants that are influencing common dichotomous traits such as type 2 diabetes, involved either hypothesis free genome wide linkage mapping in families with multiple affected subjects or association studies within candidate genes using case control samples or parent offspring trios. The linkage studies suffer from being seriously underpowered for sensible susceptibility models, because linkage is best placed to detect variants with high penetrance (McCarthy, 2003).

The candidate gene association approach historically has been compromised by difficulties associated with choosing credible candidate genes. Selection of genes was typically based on some particular hypotheses about the biological mechanisms that are putatively involved in type 2 diabetes pathogenesis, but because the function of much of the genome is poorly understood it is almost impossible to make fully informed decisions. In addition, all too often these candidate gene studies were conducted in sample sets that were far too small to offer confident detection of variants with the kinds of effect sizes that are now known to be realistic.

Rarely could the findings of one study be replicated in another study (Hattersley and McCarthy, 2005; Gloyn et al., 2003). At the same time as these GWA efforts, which had added a total of 12 loci to the list, two other loci emerged from large scale candidate pathway studies initiated in the pre-GWA era. The first of these focused on genes that were thought to be likely to modulate β-cell function and highlighted variants in the Wolfram syndrome 1 gene (WFS1) (Sandhu et al., 2007). The
second study examined variants in genes that were causally implicated in monogenic forms of diabetes and generated compelling evidence that common variants in the gene encoding hepatocyte nuclear factor 1b (HNF1B) also known as TCF2, were associated with type 2 diabetes (Winckler et al., 2007).

1.7. ADIPOQ

Adipose tissue is a fat storage tissue, but the idea has been changed in recent years as adipose tissue has emerged as an active participant in regulating physiologic and pathologic processes, including immunity and inflammation. A variety of pro-inflammatory and anti-inflammatory factors, including the adipokines leptin, adiponectin, resistin and visfatin, as well as cytokines and chemokines such as TNF-α, IL-6, monocyte chemo attractant protein 1, and others were produced by the adipose tissue (Fantuzzi, 2005). The adipose tissue was classified mainly into following two types.

- White adipose tissue (WAT)
- Brown adipose tissue (BAT)

The functional and cellular compositions are different for both the tissues. WAT or fat tissue provides insulation and by storing triglycerides serves as an energy depot. It occupies the major component of adipose tissue in the body and is dispersed in different sites like intra-abdominal (around the omentum), peri renal, intestines, buttocks, thighs and abdominal areas (Gesta et al., 2007).

The key development that established WAT as an endocrine organ was the discovery of leptin with its wide range of biological functions (Zhang et al., 1994). Earlier WAT used to be regarded as simply an energy store which also provided protection to internal organs. However, in recent
years this concept has been turned on its head and it is now recognized that WAT is highly dynamic, involved in a diverse range of physiological and metabolic processes; it has a major endocrine function secreting several hormones, notably leptin and adiponectin and a diverse range of other protein factors. These various protein signals have been given the collective name ‘adipocytokines’ or ‘adipokines’ (Trayhurn and Wood, 2004). Adipokines comprise a variety of proteins with signaling properties produced in body fat. Over fifty adipokines have already been identified with diverse structures, and can be classified according to functional role as appetite and energy balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism and homeostasis.

The brown fat which is also known as brown adipose tissue (BAT), is mostly present around the neck region. The amount of brown fat is high in hibernating animals and newborns to maintain the body temperature. The function of brown adipose tissue is to transfer energy from food into heat (Cannon and Nedergaard, 2004). Many mammals also have brown adipose tissue which stores triglyceride like white adipose tissue, but has the unique ability to generate heat. Brown adipose tissue is sometimes mistaken for a type of gland, which it resembles more than white adipose tissue. It varies in color from dark red to tan, reflecting lipid content, the lipid reserves will be depleted when exposed to a cold environment and the colour darkens.

In contrast to white fat, brown fat is richly vascularized and has numerous unmyelinated nerves which provide sympathetic stimulation to the adipocytes. In infants it comprises up to 5% of body weight, and then diminishes with age. Substantial quantities of brown adipose tissue can be detected in adult humans using positron emission tomography, especially when the individuals are exposed to cold temperatures. Intriguingly, there is an inverse correlation between the amount of brown
adipose tissue and body mass index which suggests that brown fat may be an important factor in maintaining a lean phenotype.

Similar to leptin which is produced by WAT, a novel adipose-specific protein, adiponectin has been discovered (Maeda et al., 1996; Scherer et al., 1995; Hu et al., 1996; Takahashi et al., 2000). Adiponectin gene ADIPOQ, also known as APM1, is located at chromosome 3q27 spanning about 15.7 kilo base (kb) and codes a 4.5 kb mRNA. The first exon and part of second and third exons are untranslated. Adiponectin gene has 4 distinct regions similar in sequence and structure of the C1q complement factor. It possesses a short N-terminal variable region, followed by several collagen repeats and finally a large C-terminal globular domain. Adiponectin is exclusively and abundantly expressed in white adipose tissue. The gene product is the most abundant gene transcript-1 (apM1) which codes 244-amino acid protein (adiponectin) with high structural homology to collagen VIII, X, and complement C1q as well as TNF-α (Shapiro and Scherer, 1998).

1.8. Adiponectin structure

Human adiponectin consists of 244 amino acid residues and has distinct domain structure; it contains both collagen like and globular C1q like domains. Collagen like parts of three adiponectin molecules can interact forming triple coiled coil structure much like that of collagen (Pajvani et al., 2003). C1q-like domains form “head” of adiponectin globula and share a great degree of structural similarity to complement component C1q. Several oligomeric forms of native adiponectin circulating in the blood are trimers (low-molecular weight form, LMW), hexamers (medium molecular weight form, MMW) and higher order multimers (high molecular weight form, HMW).
Structurally, based on SDS-PAGE and crystallographic studies, adiponectin appears to form a variety of higher order structures. Adiponectin monomers assemble into homotrimers with the three globular domains adjacent to one another and the three collagen-like regions forming a collagen triple helix. These trimers then assemble into hexamers and other HMW complexes.

1.9. Adiponectin function

Adiponectin as insulin-sensitizing hormone was first reported by Fruebis et al. (2001). The authors found that injection of a COOH-terminal globular adiponectin into mice acutely decreased postprandial blood glucose levels and enhanced lipid clearance via increasing fatty acid G-oxidation in skeletal muscles. This observation was subsequently confirmed and extended by several pharmacological studies using different forms of recombinant adiponectin. Yamauchi et al. (2001) demonstrated that chronic infusion of full-length or globular adiponectin produced from E. coli significantly ameliorated insulin resistance and improved lipid profiles in both lipoatrophic diabetic mice and diet-induced obese mice.

On the other hand, Berg et al. (2001) showed that intraperitoneal injection of full-length adiponectin expressed in mammalian cells triggered a significant and transient decrease in basal blood glucose levels by inhibiting the rates of endogeneous glucose production in both wild type mice and several diabetic mouse models. The chronic effects of adiponectin on insulin sensitivity and energy metabolism were also investigated in adiponectin transgenic mice or adiponectin knockout (KO) mice.

Combs et al. (2004) generated a transgenic mouse model with approximately threefold elevation of native adiponectin oligomers. The
authors demonstrated that hyperadiponectinemia significantly increased lipid clearance and lipoprotein lipase activity, and enhanced insulin-mediated suppression of hepatic glucose production, thereby improving insulin sensitivity. Yamauchi et al. (2003) showed that transgenic over expression of globular adiponectin in the genetic background of ob/ob obese mice led to partial amelioration of insulin resistance, hyperinsulinemia and hyperglycemia.

Conflicting results have been obtained from adiponectin knock out (KO) mice studies. Ma et al. (2002) found no impact of adiponectin depletion on insulin sensitivity under either normal chow or after 7 months old of feeding with a high-fat diet. In contrast, adiponectin KO mice reported by Maeda et al. (2002) exhibited that more severe high-fat diet induced insulin resistance and dyslipidemia, despite having normal glucose tolerance when fed with regular chow. Kubota et al. (2002) observed mild insulin resistance in the heterozygous adiponectin KO mice and moderate insulin resistance in the homozygous adiponectin KO mice even when fed with a regular chow. The latter two studies supported the role of adiponectin as an endogenous insulin sensitizer in mice.

The insulin-sensitizing effect of adiponectin appears to be primarily attributable to its direct actions in skeletal muscle and liver through the activation of AMP activated protein kinase (AMPK) and peroxisome proliferator-activated receptor (PPAR) (Tomas et al., 2002; Yamauchi et al., 2002). In liver, stimulation of AMPK by full-length adiponectin leads to decreased expression of gluconeogenic enzymes, such as phosphophenolpyruvate carboxykinase and glucose-6-phosphatase, which may account for its glucose-lowering effect in vivo (Combs et al., 2004; Yamauchi et al., 2002). In skeletal muscle, activation of AMPK by globular or full-length adiponectin causes increased expression of proteins, such as CD36, involved in fatty acid transport, fatty acid
oxidation (such as acyl-coenzyme A oxidase) and energy dissipation (such as uncoupling protein-2), resulting in enhanced fatty acid oxidation and energy dissipation and decreased tissue triglyceride (TG) accumulation. Excessive tissue TG accumulation has been proposed to be a major causative factor of insulin resistance in skeletal muscle (Hegarty et al., 2003). Therefore, reduction of tissue TG content by adiponectin might be the major contributor to the insulin-sensitizing activity of this adipokine.

In addition to liver and muscle, adiponectin can also act in an autocrine manner on adipocytes. It can antagonize the inhibitory effect of TNF-F on insulin-stimulated glucose uptake (Wu et al., 2003) and block the release of insulin resistance-inducing factors from adipocytes (Dietze-Schroeder et al., 2005). Furthermore, it has been suggested that adiponectin also acts in the brain to increase energy expenditure and cause weight loss (Qi et al., 2004).

1.10. Role of adiponectin in diabetes

The first reports regarding possible role of adiponectin in diabetes came from Japan in the year 2000. As adiponectin is present abundantly in the blood and having anti-atherogenic properties, plasma adiponectin level was analyzed in non-diabetic and type 2 diabetic subjects with and without coronary artery disease (CAD). The levels in the diabetic subjects without CAD were lower than those in non-diabetic subjects (respectively, 6.6±0.4 and 7.9±0.5 µg/ml in men, 7.6±0.7 and 11.7±1.0 µg/ml in women; p<0.001). The plasma adiponectin level of diabetic patients with CAD was lower than those of diabetic patients without CAD (4.0±0.4 and 6.6±0.4 µg/ml in men; 6.3±0.8 and 7.6±0.7 µg/ml in women, respectively). The presence of microangiopathy did not affect the plasma adiponectin levels in diabetic patients (Hotta et al., 2000).
Lindsay et al. (2002) found that Pima Indians who later developed type II diabetes had at baseline lower concentration of adiponectin. Individuals with high concentration of the protein were less likely to develop type II diabetes than those with low concentration which signifies that low adiponectin level is a strong predictor of future development of diabetes. Stefan et al. (2002) measured fasting plasma adiponectin and insulin concentrations and body fat composition in 30 five-year-old and 53 ten-year-old Pima Indian children. Cross-sectional, plasma adiponectin concentrations were negatively correlated with percentage body fat and fasting plasma insulin concentrations at both five and ten years of age. At age ten, percentage body fat but not fasting plasma insulin was independently associated with fasting plasma adiponectin concentrations.

In a study on the Asian Indians the mean baseline adiponectin level was lower in the diabetic subjects than the non-diabetic subjects (11.3±5.5 and 16.7±7.6 µg/ml, respectively, p=0.0017) (Snehalatha et al., 2003). Similarly, Mohan et al. (2005) reported lower adiponectin level was associated with the metabolic syndrome per se and several of its components, particularly diabetes, insulin resistance and dyslipidemia in the urban South Indian population. The Mumbai Obesity Project in western India reported that adiponectin level showed a negative association with diabetes and obesity. Proinsulin level showed an inverse association with adiponectin indicating a possible link between insulin secretion and insulin resistance (Lele et al., 2006).

1.11. KCNJ11 gene

ATP-sensitive K+ (KATP) channels couple cell metabolism to membrane excitability in various cell types, including pancreatic beta cells, neurons, endocrine cells and muscle cells. The archetypal KATP channel is an octameric complex of KCNJ11 subunits and either SUR1 subunits in
pancreatic beta cells and many neurons or SUR2 subunits in muscle. Four \( KCNJ11 \) subunits form the channel pore, and each is associated with a SUR subunit that contributes to regulation of channel gating (Girard et al., 2009).

Inagaki et al. (1995) cloned a member of the inwardly rectifying potassium channel family which was named BIR, for '\( \beta \)-cell inward rectifier,' or Kir6.2 and they determined that the \( KCNJ11 \) is intron less protein coding gene similar to other genes which encode inward rectifiers. Kir6.2 is expressed in multiple cell types that contribute to glucose homeostasis, including those involved in the release of incretins and appetite regulation (Ashcroft, 2007). By using homozygosity gene-mapping strategy in five consanguineous families of Saudi Arabian origin the gene was mapped to chromosome 11 at position 11p15.1 (Thomas et al., 1995).

**1.12. \( KCNJ11 \) and insulin secretion**

It has been widely accepted that insulin release is initiated by elevation of the intracellular \( \text{Ca}^{2+} \) concentration which is mediated by \( \text{Ca}^{2+} \) influx through voltage-gated \( \text{Ca}^{2+} \) channels in the plasma membrane. In the unstimulated \( \beta \)-cell potassium channels are open and \( \text{K}^{+} \) efflux through these channels keeps the membrane potential at a negative level where voltage-gated \( \text{Ca}^{2+} \) channels are closed (Figure 1.1A).

When the plasma glucose concentration rises, glucose uptake and metabolism by the pancreatic \( \beta \)-cell are enhanced, leading to closure of the \( \text{K}_{\text{ATP}} \) channels (Figure 1.1B). This causes a membrane depolarization that triggers opening of voltage-gated \( \text{Ca}^{2+} \) channels and \( \text{Ca}^{2+} \)-dependent electrical activity. The consequent \( \text{Ca}^{2+} \) influx stimulates insulin release (Ashcroft, 2007; Gloyn et al., 2004).
Chapter – 1

1. 13. KCNJ11 and diabetes

The earliest report regarding association of KCNJ11 SNPs with type 2 diabetes was by Thomas et al. (1996), who identified homozygote 649T-C mutation in a male infant with profound hypoglycemia. Similarly, a nonsense mutation was identified in an individual with hyperinsulinemia (Nestorowicz et al., 1997). A study conducted on Caucasians concluded that mutations in Kir6.2 are unlikely to be a major cause of NIDDM (Sakura et al., 1996). Three years later a study conducted on French population reported that homozygosity for lys23 (KK) of Kir 6.2 was more frequent in type 2 diabetics than in the control subjects (27%, 14%, respectively; p = 0.015). Analysis in a recessive model (KK vs. EK/EE) showed a stronger association of the K allele with diabetes. In the same study a meta-analysis for the E23K variant and data obtained from three other Caucasian groups found the E23K variant to be significantly associated with type II diabetes (Hani et al., 1998). In agreement with the results, the in vitro studies have implied the role of E23K polymorphism in increasing the open probability of the Kir6.2 channel, which should lead to diminished insulin secretion (Schwanstecher et al., 2002).

In a pedigree reported by Yorifuji et al. (2005), none of the patients had permanent neonatal diabetes but the members of this four-generation Japanese family, three generations showed dominant inheritance of diabetes mellitus, one member had transient neonatal diabetes, one had childhood diabetes, and the others had adult-onset diabetes without auto-antibodies or insulin resistance. In this family a novel mutation, C42R, was identified in the KCNJ11 gene and the patch-clamp experiments using the mutated Kir6.2 showed that the mutation caused increased spontaneous open probability and reduced ATP sensitivity. The effect however, was partially compensated by the reduction of functional ATP-sensitive potassium channel expression at the cell surface which
could account for the milder phenotype of patients. These results broaden the spectrum of diabetes phenotypes caused by mutations of \textit{KCNJ11} and suggest that mutations in this gene should be taken into consideration for not only permanent neonatal diabetes but also other forms of diabetes with milder phenotypes and late onset diabetes. SNPs in the active sites of Kir 6.2 can alter the KATP channel function due to the variation in the bonding affinity of ATP and the amino acid residue at these sites (Ashcroft, 2007).

Much evidence about the association of \textit{KCNJ11} mutation with type 2 diabetes came from the genome wide association studies of type 2 diabetes involving genotype data from a variety of international consortia (Diabetes Genetics Initiative of Broad Institute of Harvard and MIT and Novartis Institutes of BioMedical Research Lund University et al., 2007; Zeggini et al., 2007; Scott et al., 2007). These studies confirmed association of the E23K polymorphism (rs5219) with diabetes susceptibility. Although this association was not strongly observed in any single scan, in the meta-analyses significant association was found (OR = 1.14, \(p = 6.7 \times 10^{-11}\)).

Few studies conducted in India have evaluated the role of \textit{KCNJ11} SNPs in type 2 diabetes. Sanghera et al. (2008) who conducted their study on Sikh population inhabiting North Western part of India (Punjab) found no association between type 2 diabetes and \textit{KCNJ11} SNPs. A study conducted on Aggarwals, an endogamous caste population of North India, also reported no association of \textit{KCNJ11} SNPs with the disease (Gupta et al., 2010). In a recent study which genotyped SNP rs5219 in 5,164 unrelated Indians of Indo-European ethnicity comprising 2,486 type 2 diabetic patients and 2,678 control subjects, reported nominal association (\(p = 0.02\)) of the SNP with type 2 diabetes (Chauhan et al., 2010; Chavali et al., 2011).
On the other hand, no study on association between type 2 diabetes and \textit{KCNJ11} SNPs has been reported in literature on any South Indian population. Since the phylogenetic studies of Reich et al. (2009) and Indian Genome Variation Consortium (2008) suggested that genetic background of South and North Indian populations is different, the present study was planned to seek such an association in population of South India inhabiting Mysore district of Karnataka. For this, the coding region of \textit{KCNJ11} gene was sequenced to identify variants in the gene and evaluate the association of reported SNPs with type 2 diabetes.

\textit{1.14. Hematopoietically expressed homeobox (HHEX)}

Homeobox-containing genes are a large family of transcription factors that are distinguished by a 60-amino acid evolutionarily conserved DNA-binding homeodomain. Using somatic cell hybrid analysis and fluorescent \textit{in situ} hybridization, Morgutti et al. (2001) mapped the \textit{HHEX} gene to chromosome 10q24 and determined that the gene spans 5.7 kb with four exons.

\textit{HHEX} is expressed in the anterior definitive endoderm (Thomas et al., 1998) that gives rise to the liver and pancreas. The associated SNPs lie in a 295-kb block of LD that includes three genes, \textit{HHEX}, \textit{KIF1} and \textit{IDE} and the risk alleles have been associated with decreased pancreatic $\beta$-cell function (Pascoe et al., 2007). Throughout vertebrates, \textit{HHEX} is in conserved synteny with the neighbouring \textit{EXOC6} gene. The \textit{HHEX} conserved synteny block overlaps with a small part of the risk allele-containing LD block. This part of the LD block contains the SNPs with the highest association scores, as well as the \textit{HHEX} gene. In contrast, \textit{KIF1} and \textit{IDE} are located outside the conserved synteny block, strongly suggesting that any cis-regulatory elements within this part of the LD block regulate \textit{HHEX} and not \textit{KIF1} or \textit{IDE} (Ragvin et al., 2010).
The recent genome wide association (GWA) studies by Scott et al. (2007), The Wellcome Trust Case Control Consortium (2007) and Sladek et al. (2007) reported strong association of SNPs rs1111875 and rs5015480, located near HHEX gene region, with type 2 diabetes. In continuation to the genome wide studies, Grarup et al. (2007) validated that variants in the proximity of HHEX were associated with type 2 diabetes in Danish population. Importantly, SNPs near the HHEX confer impaired glucose and tolbutamide induced insulin release in middle aged and young healthy subjects, suggesting its role in the pathogenesis of pancreatic β-cell dysfunction. Two studies in Japanese population reported positive association of HHEX SNPs with type 2 diabetes (Horikoshi et al., 2007; Omori et al., 2008).

EUGENE2 study carried out on five European populations concluded that SNPs in the 3'-flanking region of the HHEX locus were associated with altered glucose-stimulated insulin release (Staiger et al., 2008). Though GWAs and some replication studies provided substantial evidence for the association of HHEX SNPs with type 2 diabetes, but the fact is that these two SNPs are far away from HHEX gene. Considering the above fact, Minton et al. (2007) screened all four exons (including the intron-exon junctions), the 5′ and 3′ untranslated regions and the promoter of HHEX in 30 unrelated subjects with unexplained permanent neonatal diabetes (PNDM), 32 maturity onset diabetes in young (MODY) and hypoglycaemia of infancy (n=45) and found no association of gene variants with the said phenotype.

A study in Han-Chinese of Shanghai and Beijing observed significant association between HHEX SNPs and type 2 diabetes in Shanghai (p < 0.013) but not in Beijing (p > 0.33) (Wu et al., 2008). In Norwegian population no significant association was observed for SNP rs1111875 in a study of population based sample of 1,638 patients with type 2
diabetes and 1,858 non-diabetic control participants (Hertel et al., 2008). A study based on 3,041 patients with type 2 diabetes and 3,678 control subjects of the Asian ancestry from Hong Kong and Korea reported significant association of \textit{HHEX} SNPs with type 2 diabetes (Ng et al., 2008). However, it did not observe significant association with surrogate measures of insulin secretion or insulin sensitivity indexes in a subset of 2,662 control subjects. A study conducted on 5,327 non-diabetic Finnish men to assess the impact of risk alleles on indices of insulin release, proinsulin conversion, and insulin sensitivity reported that SNP of \textit{HHEX} was nominally associated with Matsuda insulin sensitivity index (Stancakova et al., 2009).

In type 2 diabetes, the genome scans performed so far have concentrated on the European populations and have only been designed to detect those variants represented in, or tagged by, the sites on the commodity genotyping arrays. So far, a small proportion (<10%) of the overall variance in type 2 diabetes predisposition has been described.

Clearly, identification of the causal variants for the association signals uncovered will provide valuable clues to understanding disease predisposition in the Indian population. To achieve that goal, a detailed re-sequencing of the associated regions, followed by exhaustive fine-mapping in multiple ethnic groups is required. In view of the above, the present study was planned to dissect the role of three genes namely \textit{ADIPOQ}, \textit{HHEX} and \textit{KCNJ11} by sequencing their coding and conserved intronic regions in population of Mysore district in Karnataka, a South Indian state.