Chapter – 1

Introduction
INTRODUCTION

1.1 INTRODUCTION

Diabetes mellitus describes a group of multimetabolic diseases with different aetiologies, characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action or both. The Insulin deficiency and/or insulin resistance is associated with abnormalities in lipid and protein metabolism, and with mineral and electrolyte disturbances. The common symptoms of diabetes include fatigue, polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycaemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycaemia with ketoacidosis or the non ketotic hyperosmolar syndrome. Diabetes mellitus is not a pathogenic entity, but if it is not well treated, the chronic hyperglycemia results in a long-term damage and dysfunction of various cells, tissues and failure of different organs, especially the blood vessels, eyes, heart, kidneys and nerves (American Diabetes, 2011).

The pathogenic processes involved in the development of diabetes include autoimmune destruction of pancreatic β-cells with a consequent insulin deficiency to abnormalities that result in resistance to insulin action. In diabetes, the abnormalities in carbohydrate, fat, and protein metabolism arise due to deficient action of insulin on target tissues. The insulin action decreases due to inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Defects in insulin secretion and insulin action often coexist.

Complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes.

A vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process.
occurring in the pancreatic islets and by genetic markers. In the other, much more prevalent category, type 2 diabetes (T2D), the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. In the latter category, a degree of hyperglycemia is sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before diabetes is detected. During this asymptomatic period, it is possible to demonstrate an abnormality in carbohydrate metabolism by measurement of plasma glucose in the fasting state or after a challenge with an oral glucose load. The degree of hyperglycemia change over time depending on the extent of the underlying disease process (Fig. 1.1). A disease process may be present but may not have progressed far enough to cause hyperglycemia. The same disease process can cause impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) without fulfilling the criteria for the diagnosis of diabetes. In some individuals with diabetes, adequate glycemic control can be achieved with weight reduction, exercise, and/or oral glucose lowering agents. These individuals therefore do not require insulin. Other individuals who have some residual insulin secretion but require exogenous insulin for adequate glycemic control can survive without it. Individuals with extensive β-cell destruction and therefore, no residual insulin secretion require insulin for survival. The severity of the metabolic abnormality can progress, regress, or stay the same. Thus, the degree of hyperglycemia reflects the severity of the underlying metabolic process and its treatment more than the nature of the process itself (American Diabetes, 2012).

<table>
<thead>
<tr>
<th>Types</th>
<th>Normoglycemia</th>
<th>Hyperglycemia</th>
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<tbody>
<tr>
<td></td>
<td>Normal glucose regulation</td>
<td>Impaired Glucose Tolerance or Impaired Fasting Glucose (Pre-Diabetes)</td>
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<tr>
<td>Type 1*</td>
<td></td>
<td></td>
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<tr>
<td>Type 2</td>
<td></td>
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<tr>
<td>Other Specific Types**</td>
<td></td>
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<tr>
<td>Gestational Diabetes **</td>
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Figure – 1.1 : Disorders of glycemia: etiologic types and stages. *Even after presenting in ketoacidosis, these patients can briefly return to normoglycemia without requiring therapy (i.e “honeymoon” remission **), in rare instances, patients in these categories (ex. Vacor toxicity, type 1 diabetes presenting in pregnancy) may require insulin for survival. (Image adapted from ADA Criterion 2012)
1.2 CLASSIFICATION OF DIABETES MELLITUS

The major clinical manifestation of the diabetes is hyperglycemia, and the etiology of diabetes is classified into two broad categories type 1 diabetes (previously called insulin-dependent diabetes mellitus, IDDM, or juvenile-onset diabetes), T2D (previously called non insulin-dependent diabetes mellitus, NIDDM, or maturity-onset diabetes) then followed by gestational diabetes and variety of uncommon and diverse types of diabetes (American Diabetes, 2012).

I. Type 1 diabetes
   1. Immune mediated
   2. Idiopathic
II. Type 2 diabetes
III. Gestational diabetes (GD)
IV. Other specific types
   1. Genetic defects of β-cell function (American Diabetes, 2012)
      a. Chromosome 20q, HNF-4 α (MODY1)
      b. Chromosome 7p, glucokinase (MODY2)
      c. Chromosome 12q, HNF-1 α (MODY3)
      d. Chromosome 13q, insulin promoter factor (MODY4)
      e. Chromosome 17q, HNF-1β (MODY5)
      f. Chromosome 2q, Neurogenic differentiation 1/β-cell transactivator 2(MODY 6)
      g. Mitochondrial DNA
   2. Genetic defects in insulin action
      a. Leprechaunism
      b. Lipoatrophic diabetes
      c. Rabson-Mendenhall syndrome
      d. Type 1 insulin resistance
   3. Diseases of the exocrine pancreas
      a. Cystic fibrosis
      b. Fibrocalculous pancreatopathy
      c. Hemochromatosis
      d. Neoplasia
      e. Pancreatitis
      f. Pancreatectomy
4. Endocrinopathies
   a. Acromegaly
   b. Aldosteronoma
   c. Cushing's syndrome
   d. Glucagonoma
   e. Hyperthyrodism
   f. Pheochromocytoma
   g. Somatostatinoma
5. Drug or chemical induced
   a. alpha-interferon
   b. β-adrenergic agonists
   c. Diazoxide
   d. Dilantin
   e. Glucocorticoids
   f. Nicotinic acid
   g. Pentamidine
   h. Thyroid hormone
   i. Thiazides
   j. Vacor
6. Infections
   a. Congenital rubella
   b. Cytomegalovirus
7. Uncommon forms of immune-mediated diabetes
   a. Anti-insulin receptor antibodies
   b. "Stiff-man" syndrome
8. Other genetic syndromes sometimes associated with diabetes
   a. Down's syndrome
   b. Friedreich's ataxia
   c. Huntington's chorea
   d. Klinefelter's syndrome
   e. Laurence-Moon-Biedel syndrome
   f. Myotonic dystrophy
   g. Porphyria
   h. Prader-Willi syndrome
   i. Turner's syndrome
   j. Wolfram's syndrome
Type 1 Diabetes

Type 1 diabetes usually occurs in children and adolescents and is characterized by an absolute deficiency of insulin and predominantly by cellular mediated autoimmune destruction of the pancreatic islet β-cells (Expert Committee on the & Classification of Diabetes, 2003). It has an early onset, and the β-cell destruction rate is rapid in younger individuals present with ketoacidosis. The more indolent adult-onset has been referred to as latent autoimmune diabetes in adults (LADA). At the time of diagnosis, 90% of individuals have β-cell destruction, which includes antibodies to the islet cell (ICAs), to glutamic acid decarboxylase (GAD), and insulin auto-antibodies (IAAs) (P. Z. Zimmet et al., 1994). There are genetic risk factors being inherited and nearly 50% of the HLA haplotype's account for the familial clustering of type 1 diabetes. The genome wide association studies (GWAS) have identified 12 loci for this form (Cooper et al., 2008; Ounissi-Benkalha & Polychronakos, 2008). The symptoms include low or undetectable levels of serum insulin and C-peptide, hyperglycemia, ketonuria, and auto antibodies against components of the islet β-cells.

Type 2 Diabetes

T2D is the most common form of diabetes and accounts for over 90% of all diabetes cases worldwide (Gonzalez, Johansson, Wallander, & Rodriguez, 2009). T2D is often diagnosed in elderly subjects above 40 yrs of age but could occur earlier also. It’s an array of progressive disease characterized by chronic hyperglycemia and resulting from the combination of insulin insensitivity, a relative deficiency of insulin secretion and excessive or inappropriate glucagon secretion. This is the common form of diabetes accounting for more than 90% and most commonly associated with obesity, older age, family history of T2D, physical inactivity and certain ethnicities (Kuzuya et al., 2002). The symptoms of T2D develops gradually are hyperglycemia, hyperlipidemia, high serum insulin level, defective insulin secretion and insulin resistance and slow healing of wounds and sores (Kuzuya et al., 2002). T2D has a strong genetic predisposition and the common variety of T2Dis not well-defined and, at present, no specific genes have been identified in the pathogenesis of this shared metabolic disorder (DeFronzo, 1997).
**Introduction**

**Gestational diabetes**

Gestational diabetes (GD) is a form of type 2 diabetes. It usually develops during the third trimester of pregnancy. GD is defined as any alteration of glucose tolerance, hyperglycemia manifested by various degrees of severity (impaired glucose tolerance- IGT-impaired fasting glucose-IFG), arising or discovered during pregnancy with least relative deficiency of insulin secretion initially (DeFronzo, 1997; Expert Committee on the & Classification of Diabetes, 2003). The problems like altered duration of pregnancy, hypertension / pre-eclampsia, high birth weight of the newborn and placental failure may develop with GD.

**Other specific types of diabetes**

**Diseases of the exocrine pancreas**

The extensive damage to the pancreas has been associated with impaired insulin secretion leading to the development of diabetes. The most common causes are carcinoma, pancreatitis, trauma, cystic fibrosis and hemochromatosis (Tiengo, Del Prato, Briani, Trevisan, & De Kreutzenberg, 1995).

**Endocrinopathies**

The excess production of growth hormone, cortisol, glucagon, and epinephrine increase hepatic glucose production and induce insulin resistance in peripheral (muscle) tissues and can cause or exacerbate underlying diabetes (McMahon, Gerich, & Rizza, 1988). Although the primary mechanism of action of these counter regulatory hormones is the induction of insulin resistance in muscle and liver, overt T2D does not develop in the absence of β-cell failure (Werbel & Ober, 1995).

**Genetic defects**

Many genetic syndromes have been associated with diabetes mellitus, which occurs at increased frequency. Leprechaunism is a pediatric syndrome with severe insulin resistance, resulting from mutations in the insulin receptor is associated with insulin resistance (Given et al., 1980; Kahn et al., 1976). Lipoatrophic diabetes results from postreceptor defects in insulin signaling (Reitman, Arioglu, Gavrilova, & Taylor, 2000). Maturity Onset Diabetes of the Young (MODY) inherited in an autosomal dominant pattern and characterized by impaired insulin secretion with minimal or no insulin resistance (Report of the Expert Committee on the Diagnosis
and Classification of Diabetes Mellitus. 2011). Further, the inability to convert proinsulin to insulin results in mild hyperglycemia. The production of mutant insulin molecules results in mild glucose intolerance (McCarthy & Froguel, 2002). Type A insulin resistance refers to the clinical syndrome of acanthosis nigricans, virilization in women, polycystic ovaries, and hyperinsulinemia (Taylor & Arioglu, 1999).

Infections

A variety of infections have been etiologically related to the development of diabetes mellitus. Of these, the most clearly established is congenital rubella. Approximately, 20% of infants who are infected with the rubella virus at birth develop autoimmune T2D later in life. These individuals have the typical type 1 susceptibility genotype, DR3/DR4 (Menser, Forrest, & Bransby, 1978).

1.3 DIAGNOSIS

The diagnostic criteria for diabetes and pre-diabetes (intermediate hyperglycemia such as impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) have been debated for several years and modified numerous times. In 1997, the fasting glucose cut-off level was lowered from 7.8 to 7.0 mmol/l (Alberti & Zimmet 1998, Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. 2011) and in 2003, the American Diabetes Association (ADA) changed the threshold for IFG from 6.1 to 5.6 mmol/l (Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. 2011). Moreover, since 2010, ADA included the use of glycated hemoglobin (HbA1c) to diagnose diabetes and to identify individuals at “increased risk for future diabetes” (DCDM, 2011). HbA1c levels are better predictors than fasting glucose of the development of long-term complications in type 1 and T2D (UKPDS, 1998). In addition, higher levels in the sub-diabetic range have been shown to predict T2D risk and cardiovascular disease (Khaw & Wareham 2006, Pradhan, 2007). Thus, in a very recent report, the World Health Organization (WHO) as well recommended the use of HbA1c in the diagnosis of diabetes (WHO consultation, 2011). The current diagnostic criteria for diabetes and intermediate hyperglycemia according to WHO and ADA is shown in Table 1.1.
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<tr>
<th></th>
<th>Diabetes Mellitus</th>
<th>WHO</th>
<th>ADA</th>
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<tbody>
<tr>
<td>Fasting Glucose</td>
<td>≥ 7.0 mmol/l</td>
<td>≥ 7.0 mmol/l</td>
<td></td>
</tr>
<tr>
<td>Post Prandial Glucose</td>
<td>≥ 11.0 mmol/l</td>
<td>≥ 11.0 mmol/l</td>
<td></td>
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<tr>
<td>HbA1c</td>
<td>≥ 6.5 %</td>
<td>≥ 6.5 %</td>
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<tr>
<th></th>
<th>Non Diabetes Hyperglycemia</th>
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</thead>
<tbody>
<tr>
<td>Fasting Glucose</td>
<td>6.1-6.9 mmol/l</td>
</tr>
<tr>
<td>Post Prandial Glucose</td>
<td>7.8-11 mmol/l</td>
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<tr>
<td>HbA1c</td>
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The ADA, 2011 diagnostic criteria for diabetes was followed for present study (American Diabetes, 2011).

1.4 STATEMENT OF THE PROBLEM

Type 2 Diabetes (T2D) is the commonest form of diabetes constituting 90% of the diabetic population. The global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025. In recent years, the progress in understanding the etiology of T2D has been phenomenal in the developed countries, fueled by the explosion of research activities related to the identification of biomarkers and molecular genetics. However, little is known about the biochemical markers in association with genetic basis of T2D in various Indian populations. Also, several genes could be involved in the expression of the given phenotype, and it is important to examine whether or not such phenotypic expression is population-specific for understanding the issue of “heterogeneity." Hence, any genetic defects in various pathways, which are essential for glucose homeostasis, may contribute to T2D. The lipid abnormalities which occur in T2D need to be targeted, due to persistent hyperglycemia causes glycosylation of all proteins, especially collagen, and matrix proteins of the arterial wall which eventually causes endothelial cell dysfunction contributing further to atherosclerotic events occurring in people with T2D.
The relationship between the genetic and underlying biomarkers of T2DM has not been extensively studied in South Indians, although the prevalence of T2DM is very high and increasing. In explicit, its major goal is to find out DNA polymorphisms of MTNR1B, G6PC2, GCKR PPARG, IGF2BP2, CDKAL1, GCK, SLC30A8, CDKN2A/B, TCF7L2, HHEX, CDC123-CAMK1D and TCF2 genes, which plays a vital role in the process of glucose-stimulated insulin secretion and fasting plasma glucose concentrations and impact on β-cell function, which might represent the prevailing pathomechanism and how these gene variants increase the T2D risk.

Given that the Indian population diversity, the unique endogamous ethnic communities are highly suitable for epidemiologic investigation of complex diseases. The study of genetic and biomarkers risk factor assessment of T2D in South Indian populations would be truly ideal to examine the gene-gene and gene-by-environment interactions on susceptibility to T2D.

1.5 GENERAL OBJECTIVE

Keeping the above in view, the present study aims to know the influence of variations in MTNR1B, G6PC2, GCKR PPARG, IGF2BP2, CDKAL1, GCK, SLC30A8, CDKN2A/B, TCF7L2, HHEX, CDC123-CAMK1D genes and the impact of biochemical markers like fasting blood glucose, lipid profile, Adipokines and pro-inflammatory cytokines on type 2 diabetes.

Specific objectives

1. To report the phenotypic characteristics of T2D in a cohort of patients from Mysore, Karnataka State.
2. To identify the underlying biochemical factors in type 2 diabetes with special references to fasting blood glucose, lipid profile, adipokines and pro-inflammatory cytokines.
3. To evaluate single nucleotide polymorphisms within exonic or intronic regions MTNR1B, G6PC2, GCKR PPARG, IGF2BP2, CDKAL1, GCK, SLC30A8, CDKN2A/B, TCF7L2, HHEX, CDC123-CAMK1D genes and haplotypes within and between gene regions in type 2 diabetes.
4. To assess phenotype-genotype association as a validation of implication of these variants in the T2D.