CHAPTER 1
INTRODUCTION
Non-communicable diseases (NCDs) account for 80% of the disease burden globally (Haregu et al. 2015) and are one of the leading causes of death worldwide (Esteghamati et al. 2009). The four most common NCDs are cardiovascular diseases (CVD) accounting for most NCD deaths (17.5 million deaths annually), followed by cancer (8.2 million), respiratory diseases (4 million) and diabetes (1.5 million) (World Health Organization, 2015).

1.1 Diabetes Mellitus:

Diabetes mellitus is defined as a metabolic disorder characterized by chronic hyperglycemia resulting from defects in carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2014), which results into an excessive amount of glucose (≥126 mg/dl) in the blood. Patients with fasting blood glucose ≥126 mg/dl are considered “hyperglycemic” (American Diabetes Association, 2014). Diabetes mellitus has multi-factorial etiology and includes genetic and environmental elements. Sedentary lifestyle, obesity and family history of diabetes are major risk factors for development of diabetes (Yajnik et al. 1995; Yajnik, 2001).

Three main types of diabetes, such as type 1 diabetes mellitus, type 2 diabetes mellitus (T2DM) and gestational diabetes, have been identified. Type 1 diabetes is an autoimmune disease wherein the immune system attacks and destroys the β-cells in the pancreas resulting into lack of insulin. It develops most often in children and young adults. Around 5-10% diabetic patients are Type 1 diabetics (Maahs et al. 2010). T2DM is characterized by insulin resistance, impaired insulin secretion or both (American Diabetes Association, 2012). In T2DM, pancreas usually produces enough insulin, but body cannot use the insulin effectively (a condition called insulin resistance). Subsequently, insulin production decreases resulting into high glucose levels in the blood. Among global diabetic population, 85-90% patients have T2DM (American Diabetes Association, 2009). Gestational diabetes develops late in pregnancy and is caused by the hormones of pregnancy or a shortage of insulin (Engelgau et al. 1995; American Diabetes Association, 2003). Women who have gestational diabetes have 40 to 60% risk of developing T2DM within next 5 to 10 years.
There are several methods used for the diagnosis of type 2 diabetes such as oral glucose tolerance test (OGTT), random plasma glucose and fasting plasma glucose (Table 1). In OGTT, blood glucose of 200 mg/dl at 2 hours post 75g oral glucose challenge or random plasma glucose of 200 mg/dl or fasting plasma glucose of ≥126 mg/dl is regarded as diagnostic of T2DM (Wingard and Barrett-Connor, 1995). Out of these tests, the fasting plasma glucose test has been recommended globally for diagnosis of T2DM (American Diabetes Association, 2012). There are certain intermediate physiological states like impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), which show higher levels of blood glucose but the levels are not high enough to diagnose T2DM (American Diabetes Association, 2009). Impaired fasting glucose (IFG) has fasting plasma glucose between 110 and 125 mg/dl (American Diabetes Association, 2009) while impaired glucose tolerance (IGT) is characterized by 2-hr plasma glucose value of 140 or more and less than 200 mg/dl during an OGTT (American Diabetes Association, 1997).

Table 1: Criteria for Diagnosis of T2DM

<table>
<thead>
<tr>
<th></th>
<th>Fasting plasma glucose (mg/dl)</th>
<th>Oral glucose tolerance test (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>≥ 126</td>
<td>≥ 200</td>
</tr>
<tr>
<td>Pre-Diabetes</td>
<td>110-125</td>
<td>140-199</td>
</tr>
<tr>
<td>Normal</td>
<td>≤ 99</td>
<td>≤ 139</td>
</tr>
</tbody>
</table>

1.2 Prevalence of Type 2 Diabetes Mellitus (T2DM):

National guidelines and standards of care for diabetes are now available in many countries in the world. Despite this, clinical management of patients with diabetes is a challenge and remains less than satisfactory in most countries (Venkataraman et al. 2009).

1.2.1 World Scenario:

Diabetes is increasing rapidly all over the world (Whiting et al. 2011). T2DM accounts for approximately 85-90% of diabetes patients (Sicree et al. 2006) and is considered to be a major cause of morbidity and mortality (Joshi, 2003). Globally, total number of people with diabetes is projected to rise from 171 million in 2000 to
366 million in 2030, most of which will be in developing countries (Wild et al. 2004). Asia, in particular, is experiencing a rapid diabetes epidemic (Yang, 2013). India, China and U.S are the top three countries with the highest number of estimated cases of diabetes for 2000 and 2030 (Wild et al. 2004) listed in Table 2.

Table 2: List of Top 10 Countries with Highest Number of Estimated People with Diabetes in 2000 and 2030

<table>
<thead>
<tr>
<th>Rank</th>
<th>Country/Territory</th>
<th>People with diabetes in 2000 (millions)</th>
<th>Country/Territory</th>
<th>Estimated people with diabetes in 2030 (millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>31.7</td>
<td>India</td>
<td>79.4</td>
</tr>
<tr>
<td>2</td>
<td>China</td>
<td>20.8</td>
<td>China</td>
<td>42.3</td>
</tr>
<tr>
<td>3</td>
<td>U.S.</td>
<td>17.7</td>
<td>U.S.</td>
<td>30.3</td>
</tr>
<tr>
<td>4</td>
<td>Indonesia</td>
<td>8.4</td>
<td>Indonesia</td>
<td>21.3</td>
</tr>
<tr>
<td>5</td>
<td>Japan</td>
<td>6.8</td>
<td>Pakistan</td>
<td>13.9</td>
</tr>
<tr>
<td>6</td>
<td>Pakistan</td>
<td>5.2</td>
<td>Brazil</td>
<td>11.3</td>
</tr>
<tr>
<td>7</td>
<td>Russian Federation</td>
<td>4.6</td>
<td>Bangladesh</td>
<td>11.1</td>
</tr>
<tr>
<td>8</td>
<td>Brazil</td>
<td>4.6</td>
<td>Japan</td>
<td>8.9</td>
</tr>
<tr>
<td>9</td>
<td>Italy</td>
<td>4.3</td>
<td>Philippines</td>
<td>7.8</td>
</tr>
<tr>
<td>10</td>
<td>Bangladesh</td>
<td>3.2</td>
<td>Egypt</td>
<td>6.7</td>
</tr>
</tbody>
</table>

1.2.2 Indian Scenario:

India already has the largest number of people with diabetes in the world (Kaveeshwar, 2014). The prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with maximum increase for India (Wild et al. 2004). The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025 (Sicree et al. 2006). The prevalence of diabetes is 5.6% and 2.7% among urban and rural areas in India, respectively (Mohan and Pradeepa, 2009). It is reported that age standardized prevalence of diabetes and impaired glucose tolerance in urban India in 2000 was 12.1% and 14.0% respectively with no gender difference (Upadhyay, et al. 2013). Increase in the prevalence of diabetes in India is particularly attributed to “Asian Indian phenotype”.
1.3 **Asian Indian Phenotype:**

Asian Indians are a high risk ethnic group for type 2 diabetes, metabolic syndrome and coronary artery disease and have a unique phenotype called as the “Asian Indian phenotype” (Joshi, 2003; Deepa et al. 2006; Shah and Mohan, 2015) (Fig. 1). This phenotype is characterized by increased abdominal obesity and visceral fat despite low body mass index (BMI) (Raji et al. 2001; Deepa et al. 2006; Lele et al. 2006). They also have higher plasma insulin levels, insulin resistance and lower adiponectin levels (Raji et al. 2001; Deepa et al. 2006). This particular phenotype is now supposed to be particularly vulnerable to metabolic abnormalities with a significant share of insulin resistance, T2DM and CVD (Ng et al. 2008; Liu et al. 2010; Dorajoo et al. 2012).

**Fig. 1: Asian Indian Phenotype**

![Figure modified from Shah and Mohan, 2015, Current Opinion in Endocrinology, Diabetes and Obesity 22(4): 283-289.](image)

Although studies in other ethnic groups have shown that visceral adipose tissue is a major determinant of metabolic syndrome (Carr et al. 2004; Tong et al. 2007), there are none from India that have examined the association of visceral and
subcutaneous abdominal fat function with metabolic syndrome (Sandeep et al. 2010). Studies have also found that genetic polymorphisms in various genes are associated with a higher rate of diabetic complications in Indian patients (Cheema et al. 2013; Shah et al. 2013).

1.4 Risk Factors for T2DM:

Many studies have elaborated the associations between several risk factors and occurrence of T2DM. BMI, abnormal lipid profile, hypertension, sedentary lifestyle, dietary patterns, genetics are the most frequently documented risk factors for T2DM (Meisinger et al. 2002; Valdes et al. 2007; Billings and Florez, 2010; Kaur, 2014) (Fig. 2).

1.4.1 Body Mass Index (BMI):

BMI is a frequently used tool to identify the overweight and obese patients. The obesity can further be evaluated in terms of fat distribution as ‘waist to hip ratio’ (WHR). BMI is closely related to both percentage body fat and total body fat (Flegal et al. 2009). BMI, WHR and waist circumference are known as strong independent predictors of T2DM (Vazquez et al. 2007). Many longitudinal studies have reported that increased BMI is a strong risk factor for diabetes (Meisinger et al. 2002; Bays et al. 2007; Almdal et al. 2008). Various cross sectional studies have shown that higher BMI is also associated with altered lipid profile (Must and Mckeown, 2000; Tsai et al. 2004; Wild and Byrne, 2006).

1.4.2 Abnormal Lipid Profile:

Abnormal lipid profile is mostly exhibited in the form of high triglycerides, elevated low density lipoprotein (LDL) and low high density lipoprotein (HDL) levels (Mooradian, 2009). These qualitative lipid abnormalities reflect perturbations in the structure, metabolism, and biological activities of both atherogenic lipoproteins and anti-atherogenic HDL (Steiner, 2005). High blood glucose leads to increased synthesis of free fatty acids. This increased free fatty acid flux is associated with altered lipid profile which further leads to development of insulin resistance (Mooradian, 2009). Diabetic dyslipidemia is uniquely displayed in the form of elevated levels of triglyceride, LDL and decreased HDL (Steiner, 2005). Such altered
levels of lipids are known to be a risk factor for T2DM (Haffner et al. 2000; Meisinger et al. 2002). Imbalance in the levels of lipids predisposes a person to CVD and it also has a significant relation with insulin resistance (Laakso, 2010). Increased plasma triglycerides and low HDL cholesterol levels have been reported in both insulin resistance and diabetes (Taskinen, 2003; Almdal et al. 2008; Hwang et al. 2014).

1.4.3 Hypertension:

Hypertension, also known as high blood pressure (BP), is a chronic medical condition in which the blood pressure in the arteries is elevated. Blood pressure is expressed by two measurements, the systolic and diastolic pressures, which are the maximum and minimum pressures, respectively, in the arterial system. Several risk factors are responsible for the development of hypertension such as altered lipid profile, insulin resistance and BMI (Cheung and Li, 2012). Endothelial dysfunction could be one of the common pathophysiological pathways explaining the strong association between blood pressure and incidence of type 2 diabetes. Studies have shown that markers of endothelial dysfunction are associated with new-onset of diabetes (Meigs et al. 2004), blood pressure and hypertension (Hadi and Suwaidi, 2007; Kaur, 2014). Hypertensive individuals twice likely to develop diabetes than normotensive healthy people (Bonora et al. 2004).

1.4.4 Sedentary Lifestyle:

Physical inactivity is known to be a strong risk factor for development of diabetes (Almdal et al. 2008; Fretts et al. 2009). Sedentary lifestyle has been reported to be positively associated with obesity, insulin resistance and diabetes (Hu et al. 2001a, 2003) while moderate and vigorous physical activity lowers the risk of T2DM (Knowler et al. 2002; Ramachandran et al. 2006). Physical activity plays an important role in delaying or preventing development of T2DM, directly by improving insulin sensitivity and reducing insulin resistance, and indirectly by beneficial changes in body mass and body composition (Boule et al. 2001; Hamman et al. 2006). A report by Misra et al. (2011) indicates an increasing trend of sedentary lifestyle with decreased energy expenditure among Indians which has resulted in escalating obesity, dyslipidemia, T2DM and coronary heart disease in Indians.
1.4.5 Dietary Patterns:

An important life style factor associated with the risk of T2DM is dietary habits. Over the last three decades, the population is undergoing rapid nutrition transition from a healthy, traditional, high fiber, low fat, low calorie diet to a diet containing calorie dense and processed foods with edible oils, refined carbohydrates, fats, red meats and low fiber and sugar sweetened beverages (Popkin et al. 2003; Amuna and Zotor, 2008; Popkin et al. 2012).

Different patterns of food intake have recently been positively associated with risk of T2DM (Liese et al. 2009; Sun et al. 2010). Higher intake of saturated and trans-fats adversely affects glucose metabolism and insulin resistance while higher intake of polyunsaturated fats and long chain omega-3 fatty acids is known to be beneficial (Hu et al. 2001c). Consumption of food items with higher glycemic index has been consistently associated with elevated risk of T2DM in prospective cohort studies (Schulze et al. 2004). Higher consumption of white rice, butter, potatoes and whole milk is found to be associated with increased risk of T2DM while higher consumption of brown rice or other whole grains, fruits and vegetables has been shown to be associated with lowered risk of T2DM (Montonen et al. 2005; Sun et al. 2010). Insoluble fiber intake improves insulin sensitivity and thereby decreases risk of T2DM (Weickert and Pfeiffer, 2008; Lattimer and Haub, 2010). The intake of total vitamin A, C and fruits has been shown to alleviate metabolic risk factors (Park et al. 2015). Observational studies have found that low vitamin D status/intake increases the risk of T2DM (Knekt et al. 2008).

1.4.6 Genetics:

Accumulating evidence suggests that genetic factors play an important role in pathogenesis of T2DM (Harrison et al. 2003; Das and Elbein, 2006). Diabetic family history is known to increase the risk of T2DM and the risk is higher when both parents are diabetic (Meigs et al. 2000; Ma et al. 2008). Prevalence of diabetes varies substantially among different ethnic groups (Diamond, 2003), which has led to search for the genetic factors contributing to predisposition to T2DM (Das and Elbein, 2006).
1.5 Pathophysiology of T2DM:

T2DM is a disorder associated with abnormal carbohydrate metabolism which arises due to insulin resistance and impaired secretion of insulin. Insulin is a key hormone responsible for glucose homeostasis in blood (Kumar and Clark, 2002). Insulin resistance is a condition in which insulin does not exert sufficient action proportional to blood glucose concentration (American Diabetes Association, 2014). The impairment of insulin action in major target organs such as liver and muscles is a common pathophysiological feature of T2DM. Insulin resistance develops and expands prior to disease onset (Muoio and Newgard, 2008b).

Decreased insulin secretion in response to high glucose concentration in blood is observed before the clinical onset of the disease (Kohei, 2010). The patients in early stages after disease onset show an increase in postprandial blood glucose as a result of increased insulin resistance and decreased early-phase secretion (Scheen, 2003). The progression of impairment of pancreatic β-cell function subsequently leads to permanent elevation in blood glucose (Cerf, 2013). Impaired insulin secretion is generally progressive and its progression involves glucose toxicity, lipotoxicity and oxidative stress in β-cell (Groop, 2000; Prentki and Nolan, 2006). Consequent overt
hyperglycemia leads to increased synthesis of free fatty acids which accumulate in tissues and muscles, further lowering glucose uptake and insulin sensitivity (DeFronzo and Tripathy, 2009). Lipolysis is a process in which triglycerides get hydrolysed into free fatty acids, acyl-glycerides and glycerol. Increased free fatty acids and diacyl-glycerides are positively associated with insulin resistance in liver and muscles (Lara-Castro and Garvey, 2008; Jung and Choi, 2014) (Fig. 3).

Several genetic factors influencing insulin resistance (such as changes in the expressions of several genes, presence of specific single nucleotide polymorphisms (SNPs) increasing predisposition to T2DM etc.) have been identified recently (Imamura and Maeda, 2011; Prasad and Groop, 2015). SNPs of the genes like insulin receptor and insulin receptor substrate-1 (IRS-1) directly affect insulin signals while polymorphisms of genes such as β 3-adrenergic receptor and uncoupling protein (UCP) are known to be associated indirectly with insulin resistance by promoting visceral obesity (Fox et al. 2006).

Fig. 3: Pathophysiology of T2DM
1.6 Biochemical Parameters in T2DM:

1.6.1 Glucose:

Glucose is a major carbohydrate present in blood and elevated levels of blood glucose are found in diabetes mellitus. Glycemic disorders can be described as a function of 2 components: the duration and magnitude of chronic sustained hyperglycemia and the acute fluctuations of glucose over a daily period (Monnier et al. 2006). Glycemic disorders are one of the main risk factors for the development of diabetic complications (Duckworth et al. 2009), CVDs and activation of oxidative stress in diabetes (Monnier et al. 2006). High blood glucose concentration or hyperglycaemia can activate several factors including nuclear factor κβ (NFκβ), which in turn increases the expression of various genes in endothelial cells, monocyte derived macrophages and vascular smooth muscle cells (D'Souza et al. 2009). The biochemical changes induced by hyperglycaemia exert their effects through various mechanisms like, polyol pathway, activation of protein kinase C, increased oxidative stress and formation of advanced glycation end products (AGEs) which ultimately lead to development of secondary complications of diabetes (Mentink et al. 2006).

1.6.2 HbA1c:

Glycated hemoglobin (HbA1c) is a product of glycation and its concentration is proportional to the amount of glucose in the blood (Cohen et al. 2010). HbA1c provides a means for assessment of average blood glucose for past three months (Perry et al. 2001; Cohen et al. 2010). The HbA1c complex is formed when the glucose in the blood binds irreversibly (glycates) to hemoglobin. HbA1c serves as a predictor for development of diabetes and related complications (Cohen et al. 2010; Silverman et al. 2011).

1.6.3 Insulin:

The pancreatic β-cells secrete insulin in order to stimulate the glucose uptake and lower blood glucose levels (Johnson et al. 2008). Most of the glucose in blood is utilized by muscles and when muscles fail to respond adequately to circulating insulin, blood glucose levels rise (Gaziano et al. 2007). The initiation of insulin action takes place by its binding to extracellular domain of the β subunit in insulin receptor,
leading to auto-phosphorylation of several tyrosine residues in intracellular domain of the β subunit (Tatulian, 2015). Activation of tyrosine kinase of the insulin receptor, by insulin, then directs the phosphorylation of tyrosine residues of insulin receptor substrates 1, 2 and various other uncharacterized intracellular proteins (Siddle, 2012). As a result of insulin action, glucose transporters mediate the glucose transport while variety of different intracellular proteins help in metabolic and growth promoting functions of insulin (Tatulian, 2015). Insulin resistance may alter the action of insulin either at the stage of binding to the receptor or at the cellular level of downstream signaling (Kumar and Clark, 2002). Efficiency of insulin in lowering blood glucose levels is thus adversely affected in insulin resistance leading to elevated blood glucose which is known to cause adverse health effects.

1.6.4 Homeostasis Model Assessment of Insulin Resistance (HOMA-IR):

Homeostasis model assessment of insulin resistance (HOMA-IR) is a simple and reliable method for estimating insulin resistance from fasting plasma glucose and insulin levels (Gutch et al. 2015). The relationship between glucose and insulin in the basal state reflects the balance between hepatic glucose output and insulin secretion, which is maintained by a feedback loop between the liver and pancreatic β-cells (Wallace et al. 2004). It is calculated by dividing the product of fasting plasma insulin (FPI) and fasting plasma glucose (FPG), by the constant 22.5, i.e. HOMA-IR = \([\text{FPG (mmol/L)} \times \text{FPI (mU/mL)}]/22.5\]. High HOMA-IR values indicate higher insulin resistance (Matthews et al. 1985).

1.6.5 Lipid Profile:

Insulin resistance is not only associated with hyperglycemia but also with altered concentrations of lipoproteins (Krauss, 2004; Mooradian, 2009; Jung and Choi, 2014). It is well known that T2DM patients have abnormal serum lipids i.e. high levels of total cholesterol, LDL, triglycerides and lower HDL (Kim et al. 2012). The main cause of development of diabetic dyslipidemia is the increased free fatty acid synthesis (Taskinen, 2003). The increased flux of free fatty acids into the liver with adequate glycogen stores promotes triglyceride production, which in turn stimulates the secretion of apolipoprotein B (ApoB) and very low density lipoprotein (VLDL) cholesterol (Frayn, 2001). Disturbed lipid profile is a major hallmark of
metabolic syndrome and is known to play an important role in pathogenesis of CVD (Wannamethee et al. 2007). Diabetic patients have 2-4 fold higher risk for development of CVD (Daniel, 2011). Apart from glycemic management, lipid management is also an important goal in T2DM which may be achieved clinically through prescribing lipid lowering drugs such as statins (Maron et al. 2000; Barakat et al. 2013). However, statin treatment in diabetic individuals is clinically linked with various adverse effects like increase in serum glucose levels, liver function markers, cognitive loss, neuropathy, sexual dysfunction etc. (Golomb and Evans, 2008). An approach of using nutraceuticals for management of lipid profile in diabetic individuals is now attracting more attention (Alissa and Ferns, 2012). These alternative approaches have been shown to be effective in diabetic individuals in improving lipid profile and decreasing the risk of CVD (Jump et al. 2012).

1.6.6 Liver Function Test Markers:

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease characterized by lipid accumulation in hepatocytes (Paschos and Paletas, 2009). The failure of hepatocytes to respond to insulin results in altered gluconeogenesis, glycogenolysis and lipogenesis which results into hyperglycemia, dyslipidemia and insulin resistance (Harrison, 2006; Adiels et al. 2008) and consequently to diabetic liver complications (Takamatsu et al. 2008). High blood glucose, insulin resistance and high glycogen synthesis elevates liver function test (LFT) markers (Chatila and West, 1996; Hanley et al. 2005). Insulin resistance seems to promote hepatic lipid accumulation which further progresses to fibrosis in NAFLDs (Bulum et al. 2011; Smith et al. 2011). Oxidative stress due to hyperglycemia plays an important role in the compromised liver function in diabetes (Lucchesi et al. 2013; Cichoż-Lach and Michalak, 2014). Resistance to insulin, followed by oxidative stress, lipid peroxidation and damage by inflammatory cytokines is responsible for hepatic damage in NAFLD with a clinical background of hyperglycemia (Serfaty and Lemoine, 2008; Paschos and Paletas, 2009). Increasing evidence suggests that among patients with diabetes, the standardized mortality rate from end-stage liver disease (i.e., cirrhosis) is higher than that for CVD (Tolman et al. 2007).
Oxidative stress plays a major role in the pathogenesis of diabetes mellitus and acts as a mediator of insulin resistance and hyperglycemia (Tiwari et al. 2013; Tangvarasittichai, 2015). Oxidative stress, induced by excessive production of superoxide and altered antioxidant enzymes, has been linked to the development of several micro and macro-vascular complications associated with diabetes (Kesavulu et al. 2000; Giacco and Brownlee, 2010; Matough et al. 2012). Prolonged hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), which leads to cellular damage. Antioxidant enzymes provide protection against such type of oxidative damage (Tiwari et al. 2013). Free radicals affect intracellular signal transduction, gene regulation and enhance cytokine production leading to inflammatory response in the tissues (Zhu et al. 2012). Oxidative stress and changes in the activities of antioxidant enzymes like superoxide dismutase and catalase, participate in the development and progression of diabetes (Tiwari et al. 2013; Asmat et al. 2015). Several studies report lower superoxide dismutase (SOD) and catalase activities in diabetic individuals (Fujita et al. 2009, 2011; Goth and Nagy, 2013). However, there is a need to explore the association between antioxidant enzyme and insulin resistance with respect to type of treatment in diabetic individuals.

Inflammatory Marker (Interleukin 8):

Oxidative stress helps to explain the chronic low-grade inflammation in T2DM (Fuentes et al. 2013). Inflammation in diabetes is closely related to oxidative stress and failure of antioxidant defense system to scavenge the free radicals (Galassetti, 2012; Tiwari et al. 2013). Increased oxidative stress leads to increase in interleukin 8 (IL8) secretion, which in turn causes recruitment of inflammatory cells, further inducing the oxidative stress mediators, making it a key parameter in localized inflammation (Reuter et al. 2010; Kolluru et al. 2012). Inflammation contributes to the pathogenesis of atherosclerosis where IL8 acts as an atherogenic factor (Singh et al. 2002). IL8 is produced mainly by macrophages and monocytes and plays a role in modulating an inflammatory response (Straczkowski et al. 2002). Oxidized LDL particles stimulate production and secretion of IL8 by macrophages from atherosclerotic plaques (Persson et al. 2006). Serum IL8 levels are reported to be
higher in diabetic patients suggesting higher inflammation in diabetes (Esposito et al. 2003). IL8 has also been implicated in systemic insulin resistance and atherosclerosis (Srinivasan et al. 2003; Jung and Choi, 2014). Association, if any, between IL8 levels and insulin resistance is worthy of further investigations.

1.6.9 Adipocytokines:

The dysregulation of adipocytokines in abdominal or visceral obesity has been shown to participate in the development of metabolic syndrome (Matsuzawa, 2006; Jung and Choi, 2014). Adiponectin and leptin are important adipocytokines synthesized by adipose tissue. Adiponectin has a role in lipid and glucose metabolism while leptin is crucial in the regulation of metabolic activity (Maya-Monteiro et al. 2008; Richard et al. 2010). Serum leptin concentration has been shown to be related to extent of adiposity and higher leptin levels show positive association with insulin resistance (Fischer et al. 2002; Wauters et al. 2003). Lower plasma adiponectin and higher leptin levels have been reported in insulin resistant individuals (Lee et al. 2009b; Mente et al. 2010; Kim et al. 2013). Low levels of circulating adiponectin are associated with obesity, heart diseases and diabetes (Lee and Kwak, 2014b). Fasting glucose levels and BMI are inversely associated with adiponectin levels (Pham et al. 2013). Several reports suggest that the level of circulating adiponectin is a strong predictor for metabolic syndrome (Kim et al. 2013; Thanakun et al. 2014). Hence, adiponectin and leptin are considered as important biomarkers for metabolic syndrome (Ryo et al. 2004). Very few studies however reported that treatment with oral hypoglycemic agents (OHAs) like thiazolidinediones, statins and fibrates increase plasma adiponectin concentration that contribute to their beneficial effect in lowering LDL and triglyceride levels (Lin et al. 1999; Rasouli et al. 2005; Blanco-Colio et al. 2008). However, there are limited studies which have examined the effects of commonly used antidiabetic medications on adipocytokines levels in the T2DM patients.

1.7 Complications of Diabetes:

Poor management of hyperglycemia is known to affect different tissues and organs at multiple levels through different pathways involving lipid metabolism, oxidative stress, polyol pathway, glycation pathway, hexosamine pathway etc.
The long-term effects of diabetes include progressive development of microvascular complications which include retinopathy, neuropathy, nephropathy and macrovascular complications like CVD (Giacco and Brownlee, 2010) (Fig. 4). About 53.5% diabetic patients develop microvascular complications while 27.2% diabetic patients suffer from macrovascular complications (Litwak et al. 2013).

**Fig. 4: Complications of Diabetes**

![Complications of Diabetes](image)

1.7.1 Microvascular Complications:

Microvascular complications are related to small blood vessels where the basement membrane in the capillaries thickens and arterioles get affected (Cade, 2008). Diabetic retinopathy may further lead to blindness (Shaya and Aljawadi, 2007). High glomerular capillary flow coupled with hypertension and mesangial thickening leads to kidney damage resulting into diabetic nephropathy (Kanwar et al. 2011). Diabetic neuropathy is characterized by nerve dysfunction which leads to numbness, pain in foot and hands (Cade, 2008; Fowler, 2008). Both the duration and the severity of hyperglycemia are known to increase the risk of developing diabetic microvascular complications (Fowler, 2011). As many as 7% of diabetic patients are supposed to have microalbuminuria at first diagnosis of diabetes (Gross et al. 2005). The global prevalence of diabetic complications like retinopathy, neuropathy and nephropathy is as high as 26, 38 and 28% respectively (Litwak et al. 2013).
1.7.2 Macrovascular Complications:

Atherosclerotic changes leading to narrowing of arterial walls lead to macrovascular complications in diabetes (Laakso, 2010). Based on the location of atherosclerotic lesions, the macrovascular diseases are further divided into coronary artery disease and peripheral vascular disease (Cade, 2008). Atherosclerosis is thought to result from chronic inflammation, injury and accumulation of oxidized lipids in the endothelial wall of arteries (Laakso, 2010). A report suggests that hyperglycemia and lipid abnormalities are linked to the risk of CVD (Laakso, 2010). Peripheral arterial disease (PAD) is a result of atherosclerotic occlusion of arteries of legs and feet (American Diabetes Association, 2004). Potential risk factors for PAD include increased C-reactive protein, homocysteine, fibrinogen, lipoprotein and ApoB levels (Khawaja and Kullo, 2009). Consistent hyperglycemia with elevated levels of glycated hemoglobin is a well-known and independent risk factor for PAD (Cade, 2008). Prevalence of altered lipid profile is around 90% in type 2 diabetic Indian patients (Parikh et al. 2010) and hence Indian diabetics are more prone to development of CVD than other complications (Mohan et al. 2010).

1.8 Management of T2DM:

Several oral hypoglycemic agents are available for treatment of diabetes mellitus which are recommended by American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD). These drugs are administered orally and are thus also called oral hypoglycemic agents (OHAs) or oral antihyperglycemic agents. These OHAs act through different mechanisms to control blood glucose levels (Lorenzati et al. 2010). It has been suggested that consumption of healthy food and regular exercise are important lifestyle modifications for better management of blood sugar levels in T2DM. A reduction in weight and an increase in daily energy expenditure are known to decrease insulin resistance and increase glucose tolerance (Stoffers et al. 1997). Overweight patients are often advised to restrict caloric intake, consume food with low total fat content and high fibre content (American Diabetes Association, 2001).
1.8.1 Oral Hypoglycemic Agents:

Among several OHAs, metformin is considered as first-line treatment (Inzucchi et al. 2015), and thiazolidinediones (TZDs), sulfonylureas, dipeptidyl peptidase-IV (DPP-4) inhibitors, glucagon-like polypeptide-1 agonists and insulin are other options available for treatment (Nicholson and Hall, 2011). These treatments are either prescribed alone or in combination with other drugs, to achieve better effects. Treatment is based on the interplay of patient’s biochemical parameters and available therapeutic options (Stein et al. 2013). With a wide range of new pharmacological agents, the diabetes treatment has become complex and controversial with respect to their undesirable side effects (Inzucchi et al. 2015) but it has also lead to availability of more choices for clinicians for better management of hyperglycemia. Most commonly prescribed OHAs are listed in Table 3.

Table 3: Classification of Commonly Used Drugs

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Class</th>
<th>Drug</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Biguanides</td>
<td>Metformin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pioglitazone</td>
</tr>
<tr>
<td>2</td>
<td>Thiazolidinediones (TZDs)</td>
<td>Rosiglitazone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Troglitazone</td>
</tr>
<tr>
<td>3</td>
<td>Sulfonylureas</td>
<td></td>
</tr>
</tbody>
</table>
|         |                             | **First generation:**
|         |                             | Chlorpropamide    |
|         |                             | Tolazamide        |
|         |                             | Tolbutamide       |
| 4       | Alpha-glucosidase inhibitor | Acarbose          |
|         |                             | **Second generation:**
|         |                             | Glimepiride       |
|         |                             | Glipizide         |
|         |                             | Glyburide         |

Metformin is known for its anti-hyperglycemic properties and is also reported to improve lipid profile and fat redistribution (Rojas and Gomes, 2013). Metformin is also useful in chronic liver diseases (Zheng et al. 2015). Metformin treatment significantly lowers the risk for microvascular and macrovascular complications.
associated with T2DM (Kooy et al. 2009). But long-term metformin treatment lowers vitamin B$_{12}$ levels due to impaired absorption of vitamins, elevates liver function enzymes and also causes digestive disorders (Biyani et al. 2009; Bouchoucha et al. 2011; Mazokopakis and Starakis, 2012). There are very rare instances of hepatocellular and cholestatic hepatic injury (Saadi et al. 2013) and hepatotoxicity associated with metformin treatment (Miralles-Linares et al. 2012). With such excellent safety profile metformin is one of the best OHAs (Rojas and Gomes, 2013).

Other OHAs such as TZDs and sulfonylureas are also used to lower blood glucose, improve insulin sensitivity (Koppaka et al. 2013), elevate adiponectin and lower triglyceride, LDL levels in diabetes (Rasouli et al. 2005; Miller et al. 2011). TZDs have some side effects such as hepatotoxicity due to troglitazone (Della-Morte et al. 2014), increase in the risk of myocardial infarction (MI) by treatment with rosiglitazone (Nissen and Wolski, 2007), weight gain and bladder cancer (Defronzo et al. 2013). Increasing risk of MI has lead to rosiglitazone withdrawal from market in several countries including India (Nissen and Wolski, 2007).

Second generation drugs from the class of sulfonylureas enhance insulin synthesis from $\beta$-cells (Basit et al. 2012). Treatment with these drugs is observed with increased incidences of hypoglycemic episodes, weight gain and cardiovascular mortality in diabetic patients (Sarkar et al. 2011; Simpson et al. 2015). Some drugs such as alpha-glucosidase inhibitor lower glucose but they also have side effects like gastrointestinal upset and hepatotoxicity (Dabhi et al. 2013; Lee et al. 2014a).

Most of the T2DM patients frequently have adversely affected lipid profile which can be attributed to abnormal absorption, transport, synthesis and utilization of lipids (Mooradian, 2009; Miller et al. 2011). Statins are most commonly prescribed drugs for management of altered lipid profile in diabetic patients (Maron et al. 2000; Barakat et al. 2013). However, some reports show increase in the serum glucose levels after statin treatment in diabetic individuals while healthy, obese individuals have increased risk and/or incidence of diabetes (Rajpathak et al. 2009; Sattar et al. 2010; Preiss et al. 2011; Marx, 2012; Carter et al. 2013).

Several herbal medications are also used for treatment of T2DM (Patel et al. 2012). Though effective, such herbal treatments do not have established mechanism
of action since several constituents of herbal medicines are believed to act together synergistically (Vickers et al. 2001; Bandaranayake, 2006).

1.8.2 Ayurvedic Treatment:

Several herbal medications have been used traditionally for treatment of T2DM. More than 800 plants are reported to have strong antidiabetic activity (Pandey et al. 2011). Plants have a variety of phytochemicals which show diverse effects like regulation of carbohydrate metabolism, regeneration of β-cells, insulin sensitizing, improvement in glucose uptake and utilization, antioxidant properties etc. Phytoconstituents of ayurvedic drugs are believed to act in synergy with lesser side effects as compared to allopathic drugs (Patel et al. 2012). There are many herbal formulations which are used by ayurvedic clinicians as therapeutic agents to treat diabetes mellitus (Chawla et al. 2013).

1.8.3 Exercise and Weight Loss:

Regular physical activity is an important component for prevention and management of T2DM. Weight loss, especially loss of abdominal fat tissue, is an effective treatment for type 2 diabetes (Nolan et al. 2000; Boden et al. 2002). Exercise improves insulin sensitivity and lowers glucose concentrations in blood (Duncan et al. 2003). Long-term exercise reduces the amount of visceral fat and also increases glucose disposal (Thomas et al. 2000). Various studies have shown that increased physical activity has a protective effect against the development of T2DM (Boule et al. 2001; Sigal et al. 2006). Physical activity for longer time or higher intensity of exercise improves HbA1c in T2DM (Boule et al. 2001). Type 2 diabetic individuals with moderate or high aerobic fitness have 50-60% lower long-term mortality than diabetic individuals with low cardiorespiratory fitness (Wei et al. 2000; Hu et al. 2001b; Church et al. 2004).

1.8.4 Diet:

Diet modification and nutrition intervention in diabetes management have shown to improve blood glucose, lipids, blood pressure, weight etc. (Downer, 2001). Medical nutrition therapy is integral to diabetes care and management, and can be achieved by maximizing the involvement of patients in their self-management
Diet control therapy for type 2 diabetics is the most natural and safe method which achieves blood sugar at a satisfactory level (Funnell, 2006). It has been recommended that principles of dietary management of diabetes should involve a diet high in complex carbohydrates (50-60% of total intake), low in fat (<10% of total energy value), especially saturated fat, containing adequate protein (15% of total intake) and low in simple sugar (less than 25g/day) (Hosker et al. 1993; Dunning, 2003; Lattimer and Haub, 2010). Increased saturated fat consumption is said to promote weight gain in patients with T2DM and further decrease insulin sensitivity (Franz et al. 2004). A meta-analysis of low glycemic index diets also indicates significant improvement in HbA1c levels (Bantle et al. 2008).

1.8.5 Omega-3 Fatty Acids:

Recently, interest has been growing to understand the role of specific foods and nutrients in the pathogenesis of diabetes mellitus. A vast literature is available on the health effects of omega-3 polyunsaturated fatty acids (PUFA). Primarily, they control the membrane fluidity and permeability which favorably affects the conformation of cellular membranes. Besides their role in structural functions, they also act as precursors of important bioactive compounds like prostacyclins, prostaglandins, thromboxanes, leukotrienes etc. (Haggarty, 2010; Mani et al. 2011). Omega-3 fatty acid consumption has been shown to improve gene regulation and production of novel inflammation resolving mediators (Mozaffarian and Wu, 2011).

Eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) from seafood and alpha-linolenic acid (ALA, 18:3) from plant sources are the most commonly available omega-3 fatty acids for dietary intake. ALA is a cheaper plant source of omega-3 fatty acids which has potent antioxidant effects. ALA has been demonstrated to have beneficial effects in reducing blood glucose, glycated hemoglobin and cholesterol in T2DM patients (Haggarty, 2010; Mani et al. 2011). Flaxseed oil has been shown to be effective in improving cell membrane fatty acids and phospholipids which have an important role in enhancing insulin sensitivity and decreasing blood glucose in diabetic rats (El-Khayat et al. 2013). Clinical trial suggests that extract of flax dietary fiber significantly increases fat excretion and lowers total and LDL cholesterol in young individuals (Kristensen et al. 2012). Flaxseed oil supplementation has been shown to increase hepatic SOD, catalase and
decrease nitric oxide concentrations suggesting its beneficial effect in preventing tissue injury and alleviating diabetic insults in the livers of diabetic rats (Jangale et al. 2013). Flaxseed oil also helps in the prevention of diabetic complications through improvement in antioxidant defense system (Hajianfar et al. 2013). Animal studies suggest that combinational administration of flaxseed oil and trientine drug controls lipid abnormalities and oxidative stress in diabetic rats (Rezaei and Heidarian, 2013, Jangale et al. 2013).

Regulatory effect of omega-3 fatty acids on gene expression is associated with decreased homocysteine concentrations (Huang et al. 2013) thereby reducing the risk for CVDs (Kume et al. 2013). Fish oil (rich source of omega-3 fatty acids) is reported to improve plasma triglycerides in T2DM subjects (Hendrich, 2010). The beneficial effects of fish oil supplementation in improving insulin resistance, lipid profile and reducing CVD risk in T2DM has also been reviewed (Wu et al. 2012). A study from our laboratory has reported hepatoprotective effects of flaxseed and fish oil supplementation in subacute acetaminophen induced hepatotoxicity in rats (Chavan et al. 2013). Other animal studies report that diets rich in omega-3 fatty acids help to prevent visual decline in very early stages of retinopathy (Sapieha et al. 2012) and inhibit capillary cell apoptosis, vascular pathology and ameliorate retinal abnormalities (Kowluru et al. 2014). DHA rich diet has protective effect on retinopathy and improves the function of endothelial progenitor cells (Tikhonenko et al. 2013). Omega-3 fatty acids also lower microalbuminuria and HbA1c in diabetic nephropathy (Kumar and Kalaivanam, 2013b) and improve functional parameters of mitochondria through stabilization of cell membranes in diabetic rats (Zhukovs'ka et al. 2012).

However, there is a lack of evidence for these effects of omega-3 fatty acids in T2DM and the underlying molecular mechanisms have not been well evaluated (Wu et al. 2012; Devarshi et al. 2013). The available literature points to a need for studies to assess the effects of omega-3 fatty acids on lipid metabolism and its underlying mechanism (Hendrich, 2010). The use of omega-3 fatty acids as a nutritional supplement in combination with drugs may be advantageous for better management of T2DM and associated complications.
1.9 Animal Models in Diabetes Study:

Several animal models which exhibit hyperglycemia as observed in diabetic patients are available for study of diabetes. The major constraint is a lack of an animal model which mimics the human pathophysiology of T2DM. Different animal models exhibit different characteristic features such as insulin resistance, defects in glucose metabolism and altered lipid levels as observed in diabetic humans (Wang et al. 2013). They also exhibit chronic hyperglycaemia, abnormal variations in insulin levels, clinical symptoms like polyuria, polydipsia, polyphagia etc. (Velasquez et al. 1990; Kumar and Clark, 2012). With sustained hyperglycemia, animals also exhibit different diabetic complications like retinopathy, nephropathy, neuropathy, hypertension, etc. (Schafrir, 1997; McIntosh and Pederson, 1999). Diabetic animal models are usually induced (by chemicals, diet, surgery) but may be derived either spontaneously or by genetic modification of some genes that play a role in insulin resistance, obesity, hyperglycemia etc. (Srinivasan and Ramarao, 2007). These models differ significantly from each other and their advantages and disadvantages make them useful for only certain investigations (Srinivasan and Ramarao, 2007).

1.9.1 Spontaneously Induced Type 2 Diabetic Models:

Spontaneously induced type 2 diabetic animals are obtained as a consequence of one or several spontaneous genetic mutations. The metabolic peculiarities result from single gene defect in dominant (e.g. Yellow obese or KK/Ay mouse) or recessive gene (like diabetic db/db mouse, Zucker fatty rat) or it can be of polygenic origin (e.g. Kuo Kondo, New Zealand obese mouse) (Ktorza et al. 1997). Though useful, these animal models have several disadvantages. The animals are highly inbred, homogenous and display monogenic inheritance against genetic diversity as seen in humans. These animals are expensive, require sophisticated infrastructure for maintenance and they also display high mortality due to brittle pancreas, frequently requiring insulin treatment (Srinivasan and Ramarao, 2007). Diabetic animals with mutations in multiple genes resemble closely to human diabetes with multiple gene defects (Mcintosh and Pederson, 1999).
1.9.2 Diet Induced Type 2 Diabetic Models:

Diabetes can also be induced in some animals by modifying the diet with addition of specific dietary components. Sand rat, Tuco-Tuco and Spiny mouse are routinely used to induce obesity and then type 2 diabetes by providing high-fat or high-energy diet (Schafirir, 1997). These animals are characterized by marked obesity, hyperinsulinaemia, insulin resistance and glucose intolerance (Surwit et al. 1988; Velasquez et al. 1990). Because of the disadvantages like long period of dietary treatment and absence of overt hyperglycaemia, these models are not suitable for screening antidiabetic agents with effects on blood glucose levels (Srinivasan and Ramarao, 2007).

1.9.3 Chemically Induced Diabetic Models:

Chemically induced models of diabetes are common in elucidating the possible role of physiological environment in destructive processes of endocrine pancreas and subsequent development of diabetes. Hyperglycaemia develops primarily by direct cytotoxic action on the β-cells and insulin deficiency rather than consequence of insulin resistance (Tamma, 2013). Diabetes induced by chemicals is mostly less stable and at times reversible because of the spontaneous regeneration of β-cells. The chemicals used to induce diabetes may have toxic actions on other body organs as well besides its cytotoxic action on β-cells. Diabetogenic agents such as alloxan and streptozotocin are commonly used for induction of diabetes (Srinivasan and Ramarao, 2007).

1.9.3.1 Alloxan Induced Diabetic Models:

Alloxan induces diabetes through various pathways by increasing the production of oxidative free radicals and by inducing membrane destruction of pancreatic β-cells which further leads to insulin deficiency, hyperglycemia and ketosis (Battell et al. 1999; McIntosh and Pederson, 1999). Use of alloxan for inducing diabetes has many disadvantages. The percent incidence of diabetes is highly variable with high incidence of ketosis and mortality. The animals also show spontaneous reversal of hyperglycaemia due to pancreatic regeneration (Tamma, 2013). Alloxan models are most commonly used for screening anti-hyperglycemic activities of natural compounds (Tamma, 2013).
1.9.3.2 Streptozotocin (STZ) Induced Diabetic Models:

Streptozotocin (STZ) is the most commonly used drug for induction of diabetes in rats (Tamma, 2013). STZ is an antibiotic derived from *Streptomyces achromogenes*. STZ has a selective cytotoxic action on \(\beta\)-cells of pancreas. Type of diabetes and characteristics differ with the employed dose of STZ. The dose normally ranges from 60-250 mg/kg body weight and is used for destruction of \(\beta\)-cells (Srinivasan and Ramarao, 2007). Initial hyperglycemia is observed 1 hour after STZ injection followed by hypoglycemia and again a hyperglycemic state at 48 hours which remains constant thereafter (Bonner-Weir et al. 1981). There are some disadvantages associated with induction of diabetes by STZ. Animals may show spontaneous reversal to normoglycemic states and there is high incidence of tumor development in kidney and liver (Kazumi et al. 1978). STZ induced diabetic animals are most widely used for screening different compounds including natural products for their insulin mimetic, insulinotropic and antihyperglycemic activities (Tamma, 2013).

1.9.3.3 Nicotinamide-Streptozotocin (NIC-STZ) Induced Type 2 Diabetic Models:

Nicotinamide (NIC) (pyridine-3-carboxamide) is the amide form of vitamin B\(_3\). NIC ameliorates the inhibitory effect of STZ on glucose stimulated insulin secretion by isolated rat islets (Masiello et al. 1990; Bedoya et al. 1996). STZ-induced impairment in glucose oxidation and decreased islet cell viability are also markedly improved by NIC (Cheon et al. 2010). Importantly, the protective action of NIC on pancreatic \(\beta\)-cells involves a decrease in DNA damage caused by STZ (Bedoya et al. 1996). Numerous studies have also demonstrated that STZ induced increase in blood glucose is significantly reduced when NIC is administered prior to STZ (Hassan and Janjua, 2001; Su et al. 2006; Chi et al. 2007). The advantageous effect of NIC on blood glucose is due to the protection of \(\beta\)-cells against STZ-induced injury and is accompanied by increased blood insulin (Masiello et al. 1990). It is also known that STZ administered to rats diminishes pancreatic insulin content, but this effect can be prevented in a dose dependent manner by NIC administration prior to STZ (Masiello et al. 1998; Hassan and Janjua, 2001).
It is well known that streptozotocin (STZ) has a selective cytotoxic action on β-cells in islets of Langerhans whereas nicotinamide has a partial protective role on β-cells against STZ (Szkudelski, 2012), and is commonly used for induction of type 2 diabetes mellitus in experimental animals (Pari and Saravanan, 2007; Devarshi et al. 2013; Jangale et al. 2013). Additionally, NIC-STZ is considered as a suitable T2DM model to study the effects of herbal (Mohammadi et al. 2012; Maheshwari et al. 2014), and nutritional (Devarshi et al. 2013; Jangale et al. 2013) interventions.

1.10 Genesis of the Thesis:

Poor glycemic control, duration of diabetes and increased age are considered as important factors contributing to mortality in diabetic subjects (Huang et al. 2014). Several reports indicate that the risk of diabetic complications depends on the duration and severity of hyperglycemia (Fowler, 2008; Jeganathan et al. 2008; Ergul et al. 2012) and it varies with gender (Orchard et al. 1990). With increase in the duration of diabetes, the risk of developing peripheral arterial disease also increases among men (Al-Delaimy et al. 2004). Gender specific differences of the effects of T2DM have been reported by Legato et al. (2006) which indicate 4-6 fold higher risk of developing coronary artery disease in diabetic females than males. Even the pattern of diabetic dyslipidemia (low HDL, high LDL and high triglycerides) was shown to be specific for women with diabetes, predisposing them to a much higher risk of diabetes related complications than men. There are very few studies which describe gender dependent effects of T2DM and hence it is necessary to focus on sex specific effects of T2DM. Such studies may help in formulation of sex specific treatment guidelines (Legato et al. 2006; Arnetz et al. 2014). EASD/ADA guidelines recommend diabetes treatment to be individualized depending on factors such as age, life expectancy, social circumstances, presence of diabetic complications, cardiovascular risk factors, etc.

A number of classes of OHAs are currently available for better glycemic control which reduce blood glucose and improve lipid profile which in turn helps in limiting oxidative stress and inflammation (Sena et al. 2010; Rojas and Gomes, 2013; DeFronzo et al. 2014). Most of the drugs metabolizing genes show different activities in different populations, which are often major determinants of variations owing to drug exposure and response (Flockhart and Desta, 2009). In addition, drug exposure
may vary between men and women due to differences in absorption, metabolism and excretion (Huang et al. 2004; Anderson, 2005). There are very few studies examining the effect of OHAs on antioxidant enzymes and LFT markers. Moreover, there is a need to explore the association, if any, between fasting blood glucose levels, duration of diabetes, antioxidant enzymes, LFT markers, injectable insulin treatment, lipid profile and adipocytokines with the index of insulin resistance such as HOMA-IR in Indian patients. It has been reported that OHAs have heterogeneous mode of action and safety profiles (Krentz and Bailey, 2005). Many of these drugs are also reported to cause serious adverse effects (Thevenod, 2008).

Literature suggests the use of nutraceuticals and functional foods as supplementary treatment to lipid abnormalities (Alissa and Ferns, 2012; Calder, 2012; Mozaffarian and Wu, 2012). Omega-3 fatty acids are one of such recommended candidates due to their anti-inflammatory, anti-atherogenic, vasodilatory and lipid lowering properties which have been established in several chronic diseases including diabetes (Connor, 2000; Ander et al. 2003; Calder, 2012; Mozaffarian and Wu, 2012; Wu et al. 2012; Jangale et al. 2013). The lipid lowering actions of omega-3 fatty acids are attributed to regulation of key transcription factors like peroxisome proliferator-activated receptor (PPAR) and sterol regulatory element binding protein (SREBP), that control hepatic lipid metabolism (Di Minno et al. 2012). There are also some conflicting results stating that supplementation with fish oil does not delay the onset of diabetes in rats at 11 months of age (Cummings et al. 2010). Some reports suggest that omega-3 fatty acids slow the progression of T2DM and its complications (Nettleton and Katz, 2005). Moreover, the effects of omega-3 fatty acid supplementation in T2DM need to be assessed (Wu et al. 2012; Devarshi et al. 2013). A review by Hendrich (2010) points a need for studies to assess the effects of ALA. The above reports suggest the need for meticulous evaluation of effects of ALA, EPA and DHA on lipid metabolism and its underlying mechanism.

Clinical development of type 2 diabetes drugs has recently resulted in a number of new medications available to patients. Still, more progress appears to be on the horizon in the years ahead. Increasingly, multidrug therapy in type 2 diabetics has become more common using a combination of agents with different but complementary mechanisms. Despite all the new treatments that have emerged in the
last 20 years, diabetes sufferers remain challenged in effectively managing their disease over the long-term as some drugs lose efficacy and show adverse effects as the disease progresses (Inzucchi and Mcguire, 2008; Inzucchi et al. 2015). These drugs may also have different effects in men and women. Hence, it is necessary to understand gender specific differences of effects of diabetic drugs which may prove helpful in formulation of gender specific treatment.

1.11 Hypothesis:

“Type of drug treatment, fasting blood glucose levels and duration of disease may alter the lipid levels in T2DM which can be improved by omega-3 fatty acid interventions through the modulation of expression of transcription factors and genes involved in lipid metabolism and inflammation”.

In order to test the above hypothesis, the current thesis is divided into human study and animal study. The human study aimed to understand the effects of antidiabetic drugs on serum glucose, insulin, lipid profile, antioxidant enzymes, LFT markers, inflammatory marker, adipocytokines levels and its association with HOMA-IR in the diabetic male and female individuals. The present study also looked at gender dependent effects of fasting blood glucose levels and disease duration on these different biochemical markers and their association with glucose. The current study also examined expression of genes involved in lipid metabolism and inflammation from blood mononuclear cells of T2DM and healthy individuals. Based on the observations on human study, an interventional animal study was designed to understand the effects and mechanisms of omega-3 fatty acids on lipid metabolism in nicotinamide-streptozotocin induced diabetic rats. The animal study examined the comparative effects of metformin and omega-3 fatty acids on serum lipid profile, expression of transcription factors and genes involved in lipid metabolism and inflammation in diabetic rats. The objectives of the present study are listed in the next chapter.