CHAPTER I

"INTRODUCTION"
1.1. Diabetes

1.1.1. Definition

Diabetes is a group of metabolic disorders characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Expert Committee, 2003). The characteristic symptoms of diabetes mellitus include thirst, polyuria, blurring of vision, and weight loss. Ketoacidosis is the most severe forms of diabetes, a condition developed resulting into semiousness and ineffective treatment might ultimately lead to coma death. Often the symptoms of diabetes are not severe or may be absent and therefore hyperglycemia which sufficiently causes pathological and functional changes might be present for a long time even before the diagnosis of diabetes is achieved. Prolonged elevated glucose in diabetes mellitus results into progressive development of the specific complications. These complications include retinopathy leading to eye damage with potential blindness, nephropathy, a primary cause for renal failure and neuropathy with possible risk of foot ulcers, amputation, neuropathic arthritis and sexual dysfunction. Increased risk of cardiovascular, peripheral vascular and cerebrovascular diseases is associated with people affected by diabetes. The involvement of several pathogenic processes including destruction of the β-cells of the pancreas with consequent insulin deficiency and resistance to insulin action has known to be major factors in the development of diabetes. Further, due to deficient action of insulin and insulin insensitivity on target cells results in abnormalities of carbohydrate, fat and protein metabolism (Alberti et al., 1998).

1.1.2. Classification of Diabetes

Diabetes mellitus is represented by a heterogeneous group of disorders. Some distinct diabetic phenotypes can be characterized in terms of specific etiology or pathogenesis, but in many cases etiological and pathogenetic classification has become difficult due to overlapping phenotypes (Leslie, 1997). Broadly, diabetes mellitus can be classified into three main types of diabetes: (a) type 1 diabetes which results from failure in insulin secretion, (b) type 2 diabetes which is a result of insulin
resistance and (e) gestational diabetes which is observed among pregnant women, who never had diabetes before but have a high blood glucose level during pregnancy.

1.1.2.1. Type 1 Diabetes

Type 1 diabetes was formerly known as insulin-dependent diabetes mellitus (IDDM). Both adults and children were known to be affected by type 1 diabetes but, traditionally the later termed "juvenile diabetes" or "childhood-onset diabetes" as it represents a majority of the diabetes cases in children. Type 1 diabetes mellitus is characterized by destruction of the β-cells of the islets of the pancreas, with consequent insulin deficiency and is often associated with elevated blood glucose levels and symptoms including polyuria, polydipsia, and unexplained weight loss. Type 1 diabetes can be further classified as idiopathic or immune-mediated. Idiopathic refers to rare forms of diabetes with unknown cause. The majority of type 1 diabetes is of the immune-mediated nature, where β-cell loss is through T-cell mediated autoimmune attack destroying the cells in the pancreas that produce insulin (Rother, 2007). Further, the autoantibodies for glutamic acid carboxylase (anti-GAD) serve as autoimmune marker in 85-90% type 1 diabetes (Verge et al., 1996). The key symptoms of type 1 diabetes include high blood glucose levels, frequent urination, blurred vision, unusual thirst, extreme hunger, irritability and nausea, extreme hunger but loss of weight, nausea and vomiting, extreme weakness and fatigue.

1.1.2.2. Type 2 Diabetes

Type 2 diabetes was formerly known as non-insulin-dependent diabetes mellitus (NIDDM), adult-onset diabetes or obesity-related diabetes. The cause of type 2 diabetes has been often insulin resistance which may be combined with relatively reduced insulin secretion. Although patients with type 2 diabetes may have insulin levels that appear normal, however insulin levels always are relatively low compared to the elevated plasma glucose levels (Ward et al., 1984). The insulin resistance developed in type 2 diabetes is mainly because of defective insulin receptor. However, the involvement of specific defects in the insulin receptor still remains unclear. Type 2 diabetes accounts for 90 percent amongst all the types of diabetes. In the early stage of type 2 diabetes, the predominant abnormality is reduced
insulin sensitivity. At this stage hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or maintain blood glucose levels. Many people with type 2 diabetes have none of the usual symptoms and therefore remain oblivious to the problem for years, until complications begin to appear.

1.1.2.3. Gestational Diabetes

Gestational diabetes mellitus (GDM) also referred as "type 3 diabetes" resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. About 5% women suffer from gestational diabetes during the pregnancies but after delivery it has been observed that the diabetic condition may improve or disappear. Thirty to forty percent of women experiencing gestational diabetes develop type 2 diabetes within five to ten years. Gestational diabetes is fully treatable only if critical medical supervision including glucose monitoring and insulin therapy is achieved effectively throughout the pregnancy (Homko and Khandelwal, 1996). A study conducted in 2008 among American women has shown an increased number of preexisting diabetes in pregnant women. In fact the rate of diabetes in expectant mothers has been doubled in the past 6 years (Lawrence et al., 2008). The two major factors that are strongly associated with the onset of gestational diabetes are weight gain and the production of hormone resistin, responsible for altering the insulin function and signaling (Saldana et al., 2006; Kuzmicki et al., 2009). Several risks to the newborn baby including macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations might be developed by the women having gestational diabetes (Ben-Haroush et al., 2009).

1.1.2.4. Other types of Diabetes Mellitus

Besides the above mentioned three major types of diabetes, other forms of diabetes mellitus have been also observed and are classified as given below.

(a) Pre-diabetes: Pre-diabetes also known as impaired glucose regulation which includes impaired glucose tolerance (IGT) and impaired glucose fasting (IGF). Impaired glucose regulation is a metabolic condition that occurs when blood glucose
levels are higher than normal but not high enough for a diagnosis of type 2 diabetes. IGT and IGF are well characterized pre-diabetic conditions and about half of such individual’s progress to type 2 diabetes over their lifetime (Gerstein et al., 2007).

(b) Cystic fibrosis related diabetes mellitus (CFRD): Pancreatic viscous secretion is altered by abnormal functioning of the chloride channels during cystic fibrosis thereby causing obstruction of the exocrine pancreas. This mechanism might lead to the destruction of the islets in turn leading to loss of β-cells (Moran, 1991). Further, the hepatic insulin resistance with elevated glucose production is associated with the development of CFRD (Hardin, 1999).

c) Steroid induced diabetes mellitus: Glucocorticoids are employed for the therapy for bowel diseases, alcoholic and autoimmune hepatitis, and after liver transplantation. The excess or high doses of glucocorticoids results in insulin resistance developing into steroid induced diabetes mellitus. Prolonged treatment with glucocorticoid alters glucose metabolism resulting in hyperinsulinemia and increased cardiovascular risk (Kern et al., 1999).

(d) Latent autoimmune diabetes of adults (LADA): Latent autoimmune diabetes of adults also known as “type 1.5 diabetes” is a condition in which patients belongs to type 1 diabetes; although the autoimmune destruction of their islets β-cells develops often slowly (Tuomi et al., 1993; Zimmet et al., 1999). However, the processes of β-cell destruction in LADA patients are quite different, which can be discriminated by both the number of islet antibodies and the titer of glutamic acid decarboxylase autoantibody (GADA). Adults with LADA are frequently misdiagnosed as type 2 diabetes, based on age rather than etiology.

e) Malnutrition-modulated diabetes mellitus (MMDM): It was previously known as protein-deficient diabetes mellitus (PDDM). MMDM develops over a background of chronic malnutrition from childhood. MMDM patients are extremely lean and require high doses of insulin for good glycemic control (Samal et al., 2002).
1.1.3. History of Diabetes

The history of diabetes in over 3000 years old referring to the only holograph, Ebers Papyrus, which was written around 1500 BC (McFarlane et al., 1997). Ebers Papyrus was excavated in 1862 AD from ancient grave in Thebus, Egypt that describes the reference of diabetes as “too great emptying of the urine” and recommending a treatment of a liquid extract of bones, grain, grit, wheat. Nearly at the same time, Indian physicians also developed a clinical test for diabetes by observing the urine from diabetic people that attracted ants and flies and named it as “madhumeha” or “honey urine”. Approximately 1370 years later, for the first time the term “diabetes” which in Greek means “to pass through” was used by Appollonius of Mephs. In the second century a Greek physician, Arctacus of Cappadocia distinguished between diabetes mellitus and diabetes incipidus. His writings gave a detailed account of diabetes, “Diabetes is a dreadful affliction, not very frequent among men, being a melting down of the flesh and limbs into urine. The patients never stop making water and the flow is incessant, like the opening of the aqueducts. Life is short, unpleasant and painful. If for a while they abstain from drinking, their mouths become parched and their bodies dry, the patients are affected by nausea, restlessness and burning thirst, and within a short time they expire” (Zajac et al., 2002). The classification of diabetes mellitus was first time reported by two Indian ancient physicians Sushruta and Charaka in the 5th century AD that differentiated diabetes as, thin diabetics who developed diabetes at the young age and heavier diabetics, who had a late onset and lived for a long time (Papaspyros, 1964). Subsequently in the late 18th century John Rollo, Surgeon General to British Army, added the term “mellitus” to diabetes in order to distinguish it from incipidus (Rollo, 1797). In 1869, Paul Langerhans discovered that the human pancreas contained two kinds of cells, although he was unaware of their function. Some 20 years later, Josef von Mering and Oskar Minkowski found that diabetes could be induced in dogs by removing their pancreases, suggesting that this organ was somehow central to the regulation of blood sugar. In 1921, two Canadians, Frederick Banting and Charles Best, under the supervision of Professor John Macleod at the University of Toronto, injected pancreatic extract to a 14 year old boy, Leonard Thompson and observed blood glucose drop from 520mg/dl to 120 mg/dl. It was then; this anti-diabetic
substance was named as ‘insulin’ for which Banting and Macleod were awarded with a Nobel Prize for discovery of Insulin in 1923.

I.1.4. Global Burden of Diabetes

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity (Wild et al., 2004). As a primary source of information, the numerical estimates and projections for the frequency of diabetes in all countries were assembled by the World Health Organization (WHO) Ad Hoc Diabetes Reporting Group (King et al., 1998). The preliminary observations have estimated that globally, the number of people suffering from diabetes will rise from 151 million in the year 2000 (Amos et al., 1997) to 300 million by 2025 (King et al., 1998).

![Graph showing the number of people with diabetes in the adult population by region and year 1995, 2000 and 2025. Adapted from King et al., 1998.](image)

**Figure 1.1:** Number of people with diabetes in the adult population by region and year 1995, 2000 and 2025. *Adapted from King et al., 1998.*

The top three countries for estimated number of adults with diabetes between 2000 and 2025 are India, China, and the U.S.A. with India alone has 31 million diabetic people at present. The number might increase to 79 million by 2025 which is more than double (Wild et al., 2004). The major part of this numerical increase will
occur in developing countries. There will be a 42% increase in the number of diabetes adults in the developed countries and about 170% increase, in the developing countries. Thus, by the year 2025, >75% of people with diabetes will reside in developing countries. In developing countries, the majority of people with diabetes fall in the range of 45-64 years compared to the developed countries, where it is above 65 years of age (King et al., 1998).

**Table 1.1:** List of countries with the highest numbers of estimated cases of diabetes for 2000 and 2030. (*Table adapted from Wild et al., 2004*)

<table>
<thead>
<tr>
<th>Ranking</th>
<th>Country</th>
<th>People with diabetes (millions)</th>
<th>Country</th>
<th>People with diabetes (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.A.</td>
<td>17.7</td>
<td>U.S.A.</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>Japan</td>
<td>13.9</td>
</tr>
<tr>
<td>6</td>
<td>Pakistan</td>
<td>5.2</td>
<td>Pakistan</td>
<td>11.3</td>
</tr>
<tr>
<td>7</td>
<td>Russian Federation</td>
<td>4.6</td>
<td>Russian Federation</td>
<td>11.1</td>
</tr>
<tr>
<td>8</td>
<td>Brazil</td>
<td>4.6</td>
<td>Brazil</td>
<td>8.9</td>
</tr>
<tr>
<td>9</td>
<td>Italy</td>
<td>4.3</td>
<td>Italy</td>
<td>7.8</td>
</tr>
<tr>
<td>10</td>
<td>Bangladesh</td>
<td>3.2</td>
<td>Bangladesh</td>
<td>6.7</td>
</tr>
</tbody>
</table>

The global prevalence of diabetes in adults was estimated to be 4.0% in 1995 and may probably rise to 5.4% by the year 2025. Prevalence of diabetes is higher in developing countries than in developing countries. The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men, especially in developed countries (Wild et al., 2004) and diabetes will be increasingly concentrated in urban areas. Therefore diabetes in adults is now a global health problem, and populations of developing countries, minority groups, and disadvantaged communities in industrialized countries now face the greatest risk (King et al., 1993). Hence it is important to quantify the prevalence of diabetes and the number of people affected by diabetes which will allow rational planning and proper allocation of required resources.
I.1.5. Etiology of Diabetes

I.1.5.1. Obesity

Various etiological factors are responsible for the development of diabetes including obesity. Populations with the highest rate of diabetes, such as the Pima and Nauruans also have very high rates of obesity (Zimmet, 1982). Studies that have been carried for decades together suggest the increasing likelihood of development of diabetic complications according to the obesity levels. A study among women demonstrated that with increasing body mass index (BMI), the risk of developing diabetes increases (Colditz et al., 1990) and women with an average weight having a normal range of BMI were at increased risk of diabetes than does a lower BMI. Apart from BMI, waist to hip ratio (WHR) and waist circumference (WC) is also measured to determine the incidence of diabetes. In the health professional’s follow-up studies over 27,000 men higher WC, WHR, and BMI predicted development of diabetes over 13 years. The cumulative proportions of type 2 diabetes cases identified according to the medians of BMI (>24.8), WC (>94 cm), and WHR (>0.94) were 82.5%, 83.6%, and 74.1%, respectively and were 7.2 times higher than the recommended cut-offs (Wang et al., 2005).

I.1.5.2. Physical Activity and Exercise

Contracting skeletal muscle takes up more glucose from the blood during the physical activity. This effect is partly mediated by adrenaline and is positively associated with insulin sensitivity (Richter et al., 1982) which replenishes the glycogen storage thereby improving the carbohydrate metabolism during exercise. In addition, exercise has benefits in contributing to weight loss thereby influencing the development of diabetes. In a study of US male physicians, vigorous activity undertaken at least once a week led to a relatively less risk of developing type 2 diabetes, in comparison to those exercising less frequently and the effect was strongest in the most obese. (Manson et al., 1992). A similar study in 85,000 women also resulted in reducing the incidence of diabetes with regular vigorous exercise (Manson et al., 1991). Additionally, several cross-sectional studies have related the amount of time spent on watching television to the risk of having impaired glucose
tolerance and the relation has found to be significant (Dunstan et al., 2004). Indeed, the relationship appears to be stronger for television viewing time than they are for time spent undertaking physical exercise. Therefore, physical activity plays an important role in delaying or prevention of development of type 2 diabetes directly by improving insulin sensitivity and reducing insulin resistance, and indirectly by the beneficial changes in body mass and body composition (Boule et al., 2001; Hamman et al., 2006; Kay and Fiatarone, 2006).

1.1.5.3. Dietary Intake

Diet plays a very important role in the development of diabetes. However it has been remarkably difficult to pin down the precise dietary constituents that are the key players. Several studies have been carried out to understand the importance of dietary intake in the development of diabetes. A study among approximately 1300 cases was undertaken by van Dam et al., 2002 in Boston, USA. Two major dietary patterns were developed including a "prudent diet" characterized by higher consumption of vegetables, fruit, poultry and whole grains and a "western diet" characterized by higher consumption of red meat, processed meat, french fries, high-fat dairy products, refined grains, sweets and desserts. Relative risk after consumption of these diets was studied over a period of twelve years. The Western dietary pattern showed increased risk of diabetes compared to the prudent dietary pattern suggesting the association of higher dietary glycemic index with elevated risk of diabetes (van Dam et al., 2002). In addition, another study performed by Harvard School of Public Health, the high intake of red meat, low-fiber bread and cereal, dried beans, fried potatoes, tomato vegetables, eggs, cheese, and cottage cheese and low intake of wine were positively associated with markers of inflammation plasminogen activator inhibitor-1 (PAI-1) and fibrinogen that have been associated with risk of development of type 2 diabetes (Liese et al., 2009). A recent study has shown that regular consumption of white rice is associated with an increased risk of type 2 diabetes whereas replacement of white rice with brown rice or other whole grains lowers the risk of type 2 diabetes (Sun et al., 2010). The higher intake of saturated and trans fat is associated with type 2 diabetes by adversely affecting glucose metabolism and insulin resistance, (Vessby et al., 1994; Hu et al, 2001) whereas higher intake of unsaturated
Fats appear to be protective by lowering the incidence of diabetes by 25% (Salmerón et al., 2001). Another prospective study has shown higher consumption of butter, potatoes, and whole milk is associated with the increased risk of type 2 diabetes while higher consumption of fruits and vegetables was associated with the reduced risk of type 2 diabetes (Montonen et al., 2005) suggesting the possible mechanism of insoluble fiber in consistently improving insulin sensitivity and thereby decreasing the risk of the same (Salmeron et al., 1997; Meyer et al., 2000). Furthermore, large observational studies have suggested an association between low vitamin D status or low vitamin D intake and increased incidence of type 2 diabetes (Knekt et al., 2008; Pittas et al., 2006). The suggested mechanisms are attributed to vitamin D deficiency which may contribute to β-cell dysfunction, insulin resistance, and inflammation that may result in type 2 diabetes. The effect of dietary habits in all these studies has been shown to be independent of BMI change.

1.1.5.4. Hypertension

Previous prospective and case control studies have shown that progression in hypertension as an independent predictor of type 2 diabetes. A relationship of blood pressure (BP) and BP progression in the subsequent development of type 2 diabetes was shown among initially healthy women (Conen et al., 2007). The association between type 2 diabetes and hypertension has been attributed to several possible factors including endothelial dysfunction, a common pathophysiological pathway. Studies have shown the markers of endothelial dysfunction E-selectin, Intercellular Adhesion Molecule 1 (ICAM-1), and Vascular cell adhesion protein 1 (VCAM-1) were associated with the onset of diabetes (Meigs et al., 2004), and endothelial dysfunction is closely related to blood pressure and hypertension (Meigs et al., 2006). Markers of inflammation such as C-reactive protein have been consistently related to incidence of type 2 diabetes (Hu et al., 2004) and increased blood pressure levels (Blake et al., 2003), suggesting, inflammation might be another explanatory factor for the association between blood pressure, the metabolic syndrome, and incidence of type 2 diabetes (Ridker et al., 2003). Further, insulin resistance could be another potential link between blood pressure levels and the incidence of type 2 diabetes (Ferrannini et al., 1987). A relationship between hypertension and type 2 diabetes was further
strengthened by a recent randomized clinical trial showing a 14% reduction of risk of diabetes in subjects with impaired glucose tolerance and cardiovascular disease after administering valsartan, an angiotensin system blocker, over a period of 5 years (NAVIGATOR study group et al., 2010).

1.1.5.5. Smoking

Active smoking is associated with an increased risk of type 2 diabetes (Willi et al., 2007; Sairenchi et al., 2004). The risk of developing type 2 diabetes is 2.1 times more in smokers consuming more than 20 cigarettes per day when compared with non-smokers. Therefore cigarette smoking is an independent and a modifiable determinant of type 2 diabetes mellitus (Manson et al., 2000). Further, prolonged smoking results into development of insulin resistance and inadequate compensatory insulin secretion response (Attvall et al., 1993; Facchini et al., 1992). This effect could be due to a consequence of nicotinic components of cigarette smoke on β-cells of the pancreas as which is known to be associated with chronic pancreatitis and pancreatic cancer (Talamini et al., 1999). Although cigarette smoking predicts incident of type 2 diabetes, but smoking cessation is associated with weight gain and leads to higher short-term risk (Willi et al., 2007; Yeh et al., 2010). Therefore, for smokers who are at a risk for diabetes, smoking cessation should be paired off with certain schemes involving early detection and prevention of diabetes. However, a follow up over a longer period of time have shown a reduction in risk of development of type 2 diabetes after smoking cessation (Wannamethee et al., 2001).

1.1.5.6. Ethnicity

According to the 2000 census data ethnic minorities constitute approximately 25% of the overall population of the United States (Anonymous, 2012). For example, in comparison with the prevalence rate of diabetes in white Americans, the relative increase in the prevalence of type 2 diabetes is 10 fold greater in certain Native American ethnic groups (Burke et al., 1999; Flegal et al., 1991). In the last fifteen years the increase in prevalence of diabetes has been high (about 68%) among Asians (McBean et al., 2004). The prevalence of diabetes among urban population in India is 20% compared to rural population which is about 10% and is mainly attributed to
sedentary lifestyles among the urban population (Ramachandran et al., 1999). Diabetes prevalence substantially observed among ethnic groups could be attributed to negligence for care along with several other socioeconomic factors aggravating the unequal burden of diabetes among minority populations.

1.1.5.7. Family History

Risk factors for type 2 diabetes are well established and include underlying genetic susceptibility which is reflected by family history. Genetic components play an important role in pathogenesis of type 2 diabetes (Amini and Janghorban, 2007). Diabetic family history among first degree relatives confers an increased risk of developing type 2 diabetes and the risk is even greater when both parents are diabetic (Bjornholt et al., 2000; Ma et al., 2008). Both maternal and paternal transmission of diabetes significantly contributes and influences the risk of developing diabetes in offsprings. Among Indian population, there is increased risk of developing diabetes increases two- to fourfold for an individual with a positive family history of diabetes (Padaki et al., 2011). Very high risk for abnormal glucose homeostasis among offspring with young age-of-onset maternal diabetes is observed (Meigs et al., 2000). Therefore, globally evaluation of family history could be a promising new public health tool to fight against the growing epidemic of diabetes.

1.1.5.8. Genetics

Over a past decade, there has been a spectacular change in the capacity to identify common genetic variants that contribute to the development of diabetes (Swapan and Stevan, 2006). Gene variants including transcription factor 7-like 2 (TCF7L2) (Grant et al., 2006), peroxisome proliferator-activated receptor gamma (PPARG) (Altshuler et al., 2000), fat mass and obesity-associated protein (FTO) (Frayling et al., 2007), KATP channel subunits Kir6.2 (KCNJ11) (Gloyn et al., 2003b), NOTCH2, wolframin (WFS1) (Saxena et al., 2007), CDK5 regulatory subunit associated protein 1-like 11(CDKAL1), insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2), solute carrier family 30 member 8 (SLC30A8), juxtaposed with another zinc finger protein 1 (JAZF1) and hematopoietically-expressed homeobox protein (HHHEX) has significantly associated with the risk of type 2 diabetes. These
eight genes are also associated with impaired β-cell function and are independent of other clinical risk factors (Lyssenko et al., 2008a). Most of these gene variants are responsible for reducing insulin sensitivity and insulin secretion among most of the diabetes cases (Elbein et al., 2000; Gerich et al., 1998). The expression of TCF7L2, the transcription factors 7-like 2, is the locus with the highest risk of type 2 diabetes amongst all the variants (Lyssenko et al., 2008b) corresponding to approximately 25% of risk, due to an average single allele frequency 18-30% in Northern Europeans (Cauchi et al., 2008). Through various efforts, approximately 20 common variants are now robustly implicated in type 2 diabetes susceptibility (Prokopenko et al., 2008). According to James Neel’s ‘thrifty gene’ hypothesis, postulating that such genes allow efficient food utilization, fat deposition and rapid weight gain in times of plenty, in preparation for famine, thereby making the gene-bearer to survive in a subsequent famine (Neel, 1962). Such genes would be advantageous for traditional human lifestyle, but they would lead to obesity and diabetes in the modern world when the same individuals lack physical activity. Apart from genes predisposing to type 2 diabetes, further investigation is required to define the causative variants thereby understanding the basis of disease mechanisms which is known to affect the pancreatic β-cell function leading to the development of type 2 diabetes.

1.1.5.9. Sociocultural Factors

Apart from the biomedical risk factors that are involved in the development of diabetes, sociocultural factors have also known to play a significant role. The impacts of urbanization have changed many traditional lifestyles into modern lifestyle by altering the dietary habits of the population and therefore inclining towards the development of type 2 diabetes. In a recent study of the Cambodian population, the prevalence of diabetes was twice in the semi urban community as compared to the rural community (King et al., 2005). Another Chennai Urban Rural Epidemiology Study which screened 26,000 individuals, the prevalence of diabetes among the urban Indian population was more than the rural counterparts. Also, a cross-sectional population survey undertaken by Diabetes India group has revealed that the prevalence of diabetes in urban population is higher compared to rural population (Sadikot et al., 2004). A negative correlation has been observed between
socioeconomic status and type 2 diabetes population from United Kingdom. However, the increased prevalence of diabetes is associated with people living in the most deprived areas (Connolly et al., 2000). The possible explanation for this observation could be lack of information about healthy food and complete dietary food practice due to deprived access to health information. On the contrary, in developing countries the impact of poverty has known to decrease the prevalence of diabetes. A study involving south India and China, the higher income groups were twice as likely to have diabetes as the lower income group (Ramachandran et al., 2002). In the developing countries, poor people are involved in manual work and therefore have limited chance to develop diabetes. Moreover, rural population practice traditional food, which comprises of more fruit and vegetables helping in lowering the risk of development of diabetes than the wealthier population with ready access to westernized food (Ekoe et al., 2008).

1.1.6. Screening Detection and Diagnosis of Diabetes

Diabetes mellitus is a common and serious disease that has widely affected the global population where about 30% of the affected population is unaware of being affected (Centres for Disease Control and Prevention, 1997; Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). During clinical diagnosis of diabetes, the individual is frequently observed with several risk factors for macro as well as microvascular complications (Harris and Modam, 1994; Whiteakar et al., 1997). Most of the times the onset of diabetes may occur about 9-12 years before the diagnosis were made by the medical practitioner (Harris et al., 1992). Therefore early detection of type 2 diabetes through screening may be an appropriate public health strategy to prevent or delay diabetic complications and improve health outcomes (Pauker, 1993). A major distinction between diagnostic testing and screening is elucidated in Table 2. When an individual exhibits symptoms or signs of the disease, diagnostic tests are performed whereas the purpose of screening is to differentiate an asymptomatic individual at high risk of an individual at low risk for diabetes (Engelgau et al., 2000).
Table 1.2: Proposed criteria for screening and diagnosis of diabetes. FPG, fasting plasma glucose; A1c, Glycated hemoglobin; RPG, random plasma glucose; OGTT, Oral Glucose Tolerance Test. (*Table adapted from Saudek et al., 2008*)

<table>
<thead>
<tr>
<th>Screening</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPG ≥ 100 mg/dl</td>
<td>FPG ≥ 126 mg/dl</td>
</tr>
<tr>
<td>HbA1c &gt; 6.0%</td>
<td>HbA1c &gt; 6.5%</td>
</tr>
<tr>
<td>RPG ≥ 130 mg/dl</td>
<td>RPG ≥ 200 mg/dl</td>
</tr>
<tr>
<td>If a screening result is negative, screen again in 3 years</td>
<td>2-h OGTT ≥ 200 mg/dl</td>
</tr>
<tr>
<td>If a screening result is positive but below the diagnostic threshold, perform another test for diagnosis, using different method</td>
<td>Diagnosis requires confirmation unless unequivocal symptoms of hyperglycemia are present</td>
</tr>
<tr>
<td>If a screening result is above the diagnostic threshold but the second test does not reach threshold, test again in 1 year</td>
<td>Diagnosis based on HbA1c requires confirmation using glucose dependant test (FPG or OGTT) or, if HbA1c is ≥ 7%, by a second HbA1c ≥ 6.5%</td>
</tr>
</tbody>
</table>

The World Health Organization (WHO) recommends following revised criteria for diagnosis of diabetes mellitus:

1. Fasting plasma glucose (FPG) concentration is greater than or equal to 7 mmol/L (140 mg/dL).
2. Postprandial 2-h plasma glucose concentration is greater than or equal to 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (OGTT) (Alberti et al., 1998).
3. Symptoms of diabetes and a casual (i.e., regardless of the time of the preceding meal) plasma glucose concentration greater than 11.1 mmol/L (200 mg/dL).
4. Hemoglobin A1c (HbA1c) greater than 6.5% (48 mmol/mol).

If one of the above criteria is met, confirmation of the test is required by repeating the test on subsequent days. In 2009, the International Expert Committee (The International Expert Committee, 2009), which comprises of members appointed by the American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD), and the International Federation of Diabetes (IDF), recommended that diabetes can be diagnosed by measurement of HbA1c, which reflects long-term blood glucose concentrations. The ADA and the WHO have certified the use of HbA1c for diagnosis of diabetes (American diabetes association,
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2010). Moreover, the annual testing for albuminuria is recommended by all major
guidelines for diabetic patients where the level of urine albumin level (corresponding
to 30mg albumin/g of creatinine) is used for determining the rate of disease
progression in diabetes particularly in end-stage renal disease (Sacks et al., 2011).
Apart from the all above parameters, measurement of insulin and islet cell antibodies
from the blood can be used for diagnosis of diabetes only after standardizing the
prospective clinical studies. Similarly, evaluation of insulin and C-peptide
concentration in blood and ketone bodies in urine also requires further prospective
studies in order to consider as a diagnostic marker for diabetes.

1.2. Diabetes and Glycation

Diabetes is characterized by hyperglycemia having plasma glucose levels
more than 200 mg/dl. Prolonged exposure of plasma proteins to the elevated blood
glucose has been observed in diabetic patients with poor glycemic control (Austin et
al., 1987). Several plasma proteins including hemoglobin, serum albumin and
transferrin have shown glycation, a post translational modification (PTM) caused by
non-enzymatic reaction between glucose and protein (Ulrich and Cerami, 2001).
Glycation is a chemical modification of proteins where condensation between the
carbonyl group of glucose and free amino group of protein leads to the formation of
the Schiff’s base. This is the first of step glycation reaction, the formation of the
Schiff base from sugar and amine is relatively fast and highly reversible (Ulrich and
Cerami, 2001). The next step involves conversion of thermodynamically unstable
Schiff’s base into a stable reversible Amadori product. Proteins bearing Amadori
product are referred as glycated proteins. Finally, the Amadori product undergoes a
series of dehydration and fragmentation reactions and results into a variety of
carbonyl compounds including methylglyoxal, glyoxal, glucosones, 3-
deoxyglucosone (3-DG) and so on (Thornalley et al., 1999). These carbonyl
compounds are more reactive than the original sugar and act as propagators of
reaction leading to the formation of advanced glycation end products (AGEs). The
mechanisms involved in glycation and the formation of AGEs are illustrated in Fig
1.2.
Glycation reaction preferentially takes place at epsilon amino groups of amino acids including lysine, arginine and histidine; however, crosslinking of AGEs with protein is more specific at arginine (Münch et al., 1999). Different modifications have been reported on glycated proteins and have been characterized by mass spectrometric analysis. For example, as shown in the Table 1.3 glycation modification at lysine residue shows increase in mass of 162.0258 Da. Several other modifications like carboxymethyllysine (CML), carboxyethyllysine (CEI) and pyrraline have been reported in the previous studies (Fig 1.3) (Wa et al., 2007; Thornalley, 1990; Biemel et al., 2001). Protein glycation has strongly decreased the activity of hexokinase and glucose-6-phosphate dehydrogenase (Kiho et al., 1996). The key antioxidant enzymes, glutathione peroxidase and glutathione reductase are inactivated by glycation reaction (Vander, et al. 1997; Niwa and Tsukushi, 2001). Further, AGEs have known to interact with the amino groups of several other proteins including collagen thus resulting in the formation of protein cross-links (Reddy, 2004).

Quantitative screening of a comprehensive range of AGEs, have shown that intracellular proteins are heavily glycated than extracellular proteins due to higher amount of intracellular AGEs (Thornalley et al., 2003b). The probable reason of
increase in intracellular AGEs is the auto oxidation of glucose resulting in the formation of either glyoxal or deoxyglucosone or methylglyoxal (Wells-Knecht et al., 1995; Thornalley et al., 1999). Three major mechanisms are known for the damage of the target cells due to production of AGEs. 1) AGE modification of intracellular proteins alters their function, 2) the extracellular matrix components and 3) AGE modified plasma protein binds to the receptor for AGE (RAGE) thereby inducing receptor-mediated production of reactive oxygen species (ROS) (Brownlee, 2001).

AGEs are substantive contributors to the progression of diabetic complications. If the glycation site of a protein occurs close to the protein’s active sites or if stereochemical configuration is disturbed, then the protein function is known to be altered (Taylor and Agius., 1988). The involvement of several glycated proteins has been implicated in the development of glycation induced complications in diabetes. Glycated fibrin has shown lesser susceptibility for fibrin digestion (Brownlee et al., 1983), thereby resulting into permanent vascular occlusion. Glycation of several lipoproteins has affected the lipid transport in diabetes for example; glycated apolipoprotein B causes a reduction of affinity for the low density lipoprotein receptor (Kesaniemi et al., 1983). Similarly glycation of apolipoprotein A-1 altered its structure thereby adversely affecting the reverse cholesterol transport in diabetes, thus, developing a risk for cardiovascular diseases (Nobecourt et al., 2007). The collagen glycation results in increased intramolecular cross-links causing the decreased small joint mobility of longstanding diabetes (Schnider and Kohn, 1982).
Figure 1.3: Structural formulae of different Advanced Glycation End products (AGEs). Modified from Wa et al., (2007); Lapolla et al., (2006); Biemel et al., (2001)
Table 1.3: Glycation modifications with mass increase (ΔM) for different AGEs.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Abbreviations</th>
<th>Modification</th>
<th>ΔM (Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FL</td>
<td>Fructosyl-lysine</td>
<td>162.0528</td>
</tr>
<tr>
<td>2</td>
<td>CEL</td>
<td>N\textsubscript{ε}-carboxyethyl-lysine</td>
<td>72.0211</td>
</tr>
<tr>
<td>3</td>
<td>CML</td>
<td>N\textsubscript{ε}-carboxymethyl-lysine</td>
<td>58.0055</td>
</tr>
<tr>
<td>4</td>
<td>PYRALINE</td>
<td>Pyrraline</td>
<td>108.0211</td>
</tr>
<tr>
<td>5</td>
<td>FL-2H\textsubscript{2}O</td>
<td>Fructosyl-lysine- 2H\textsubscript{2}O</td>
<td>126.0317</td>
</tr>
<tr>
<td>6</td>
<td>IMIDAZOLONE-A</td>
<td>Imidazolone-A</td>
<td>144.0300</td>
</tr>
<tr>
<td>7</td>
<td>IMIDAZOLONE-B</td>
<td>Imidazolone-B</td>
<td>142.0266</td>
</tr>
<tr>
<td>8</td>
<td>ARGPYR</td>
<td>Argpyrimidine</td>
<td>80.0262</td>
</tr>
<tr>
<td>9</td>
<td>MG-H1</td>
<td>Ne-(5-hydro-5-methyl-4-imidazolon-2-yl)ornithine</td>
<td>54.0106</td>
</tr>
<tr>
<td>10</td>
<td>G-H1</td>
<td>Ne-(5-hydro-4-imidazolon-2-yl)ornithine</td>
<td>39.9949</td>
</tr>
<tr>
<td>11</td>
<td>AFGP</td>
<td>1-alkyl-2-formyl-3,4-glycosyl-pyrrole</td>
<td>270.0740</td>
</tr>
<tr>
<td>12</td>
<td>MOLD</td>
<td>2-ammonio-6-[1-(5-ammonio-6-oxido-6-oxohexyl)-4-methylimidazolium-3-yl]hexanoate</td>
<td>49.0078</td>
</tr>
<tr>
<td>13</td>
<td>CROSSLINE</td>
<td>Crossline</td>
<td>252.1100</td>
</tr>
</tbody>
</table>

A recent study shows glycation of two central extracellular matrix (ECM) proteins laminin and fibronectin associated with neuronal regeneration are directly linked to the failure of axonal regeneration and are therefore involved in the development of diabetic neuropathy (Duran-Jimenez et al., 2009). Glycated albumin is known to inhibit hepatic uptake of glycoproteins (Summerfield et al., 1982) and is also responsible for stimulating renal extracellular matrix production thereby associating with the development of diabetic nephropathy (Chen et al., 2000). The levels of AGEs are correlated with initiation and progression of diabetic retinopathy which is associated with retinal capillary basement membrane thickening, blood retinal barrier dysfunction and loss of pericytes (Stitt et al., 2000).

As glycation is implicated in the development of diabetic complications by structural and functional modification of several proteins there is substantial need to understand the mechanism of protein glycation and their implication in developing diabetic complications.
I.3. Glycation and Plasma Proteome

I.3.1. Plasma Proteome

Blood plasma is not only an exceptional primary clinical specimen but is also the most complex and deepest version human proteome, containing other tissue proteomes as subsets. It possesses rich information concerning the overall pathophysiology of the patient as is in close association with the body. Blood plasma represents an organ or tissue status in health and disease condition due to the presence of significant fractions of tissue-derived proteins as a part of plasma proteome (Zhang et al., 2007). Plasma proteome comprises of true plasma proteins that carry out their functions in the circulation, tissue leakage proteins which are released into plasma as a result of cell death or damage and immunoglobulin class of proteins with tremendous sequence heterogeneity involved in generating their functional specificities (Anderson and Anderson, 2002). There is a large difficulty to obtain other body fluids or tissue sections involving painful and risky invasive procedure in comparison with blood plasma which is readily accessible. Moreover, blood plasma is in circulation throughout the body suggesting that plasma proteome may serve as a treasure trove of protein biomarkers (Issaq et al., 2007). Diagnosis of the involving vascular, renal, retinal, and neural complications in diabetes is quite possible, due to the presence of tissue leakage proteins in plasma of diabetic patients. In addition, the accumulation of glycated proteins has been strongly implicated in the progression of glycation induces diabetic complications which itself is a prerequisite for understanding plasma protein glycation in greater detail.

I.3.2. Dynamic Range of Plasma Proteome

The complexity and dynamic range of specific protein concentration in human plasma spans greater than ten orders of magnitude. This reduces the ability to discover highly specific tissue-derived biomarkers which are present in very low concentration (about ng/ml) (Whiteaker et al., 2007). Twenty two proteins including albumin, IgG, IgA, haptoglobin, alpha-1-antitrypsin and transferrin constitute about 99% of the protein total plasma concentration, while the remaining 1% considered being at low abundant proteins (Tirumalai et al., 2003). It is within this 1% of the plasma proteome
that many tissue secreted proteins are present and are dominated and masked by high abundant proteins.

Although reverse phase LC-MS/MS analysis would certainly be helpful in identifying low abundant proteins but may not be sufficient enough to gain comprehensive coverage of the low abundant plasma proteins. Therefore, effective characterization of plasma proteins requires several methods involving removal of the high abundance proteins prior to downstream analysis.

1.3.3. Abundant Protein Depletion of Plasma

Up to 10,000 proteins might be present in plasma with varying concentrations from millimolar to femtomolar amount (Issaq et al., 2007). Albumin, the most abundant protein and twenty one other high abundant proteins in plasma represent a major challenge for the proper detection of other low abundant proteins (Fig 1.4). For this reason, it is important to unmask the low abundant proteins by various approaches. Albumin can be depleted from plasma by using a dye based approach, Cibracon blue, which has higher affinity towards albumin (Zolotarjova et al., 2005).

Even though abundant protein depletion reduces the dynamic range of the plasma proteome by about 2-3 orders of magnitude, the difference between medium-abundant and low abundant plasma proteins is still in the range of 7-8 orders of magnitude and beyond the dynamic range of current proteomic technologies (Linke et al., 2007).

Therefore exploring the plasma proteome in depth still remains a difficult task as we are unaware about the proteins that are below the tip of the iceberg. This technical difficulty can be overcome by using a multiple affinity removal system (MARS) an immunodepletion column for removal of six high abundant proteins in plasma (Dardé et al., 2007). Similar antibody based approaches involving immunoaffinity columns are more specific in removing either 12 (IgY-based affinity LC column, Agilent) or 20 (Prot20, Sigma) most abundant plasma proteins (Linke et al., 2007), thus facilitating in-depth proteomics analysis. In addition to these immunoaffinity columns, development of the ProteoMiner technology which includes hexapeptide ligand library further serves in capturing the 'hidden proteome' thereby equalizing the plasma protein concentration (Righetti et al., 2006).
CHAPTER 1. INTRODUCTION

Proteomics: A Tool for Studying of Plasma Protein Glycation

Proteomics is the systematic and comprehensive study of the diverse properties of proteins in a parallel manner with the aim of providing detailed descriptions of the structure, function and control of biological systems in health and disease (Patterson and Abersold, 2003). There is a substantial need for studying the human proteome because the genome is more or less constant, but the protein expression might differ according to cell location and time (Dhingra et al., 2005). Proteins are virtually the effectors of biological functions, but protein expression not only depends on the levels of the corresponding mRNA but also on a host of translational controls and regulated degradation (Gygi et al., 1999). Although transcriptome analysis might help in identification of differentially expressed genes, such techniques fail to detect post-translational modifications of proteins, which are significantly crucial in determining specific cellular process (Ohtsubo et al., 2005). In this regard, proteomics is mandatory for the better understanding of cellular function thereby elucidating molecular mechanisms in health and disease. Proteomics

Figure 1. 4: Plasma proteome. Albumin accounts for over more than 50% of the plasma proteome with IgG is the second most abundant.
preliminary aims in the simultaneous measurement of levels of large numbers of proteins obtained from complex biological samples (Sundsten and Ortsater, 2008) and initially pursued with proteome separation by two dimensional gel electrophoresis (2DE), protein identification MS followed by immunoblotting for protein validation. However, the technology was not efficient enough to deal with analysis of PTMs. Therefore, biological mass spectrometry (MS), the technological basis of most current proteomics studies, was launched with the development of two major ionization techniques involving the electrospray and Matrix associated laser desorption ionization (MALDI) for identification of protein and characterization of PTMs (Cox and Mann, 2007; Lapolla et al., 1998).

1.4.1. Proteomics and PTM analysis

Previously identification of PTMs used to be achieved by Edman degradation, amino acid analysis, isotopic labelling, or immunochemistry. During the last one and half decade mass spectrometry based proteomics has emphatically become a formidable tool for detecting post translational modifications which of course could not be predicted from genomic information alone. Mass spectrometry has several advantages for PTMs characterization, including (i) very high sensitivity; (ii) ability to identify the exact site of PTM; (iii) discovery of novel PTMs; and (iv) capability to identify PTMs in complex protein mixtures (Cox and Mann, 2007). With the advent of new techniques, such as electron transfer dissociation (ETD), which preserves modifications better than collision dissociation (CID) due to differences in the manner of fragmentation, the analysis of ‘difficult’ PTMs has been significantly improved (Mitchell et al., 2010). Applied to disease proteomics, the latest mass spectrometric techniques involving MALDI-MS and nano-ESI-LC-MS, have proved highly effective in shedding light on the glycation induced PTMs in diabetes (Lapolla et al., 2011). The use of mass spectrometry based proteomics in revealing the post translational modification in diabetic complications including glycation will expand substantially, particularly to meet the need for better diagnostics and to shorten the path for developing effective therapy.
1.4.2. Strategies for Characterizing Plasma Protein Glycation

Protein glycation modifications can be characterized by using various separation methods including ion-exchange chromatography (Al-Abed et al., 1999), capillary electrophoresis (Fayle et al., 2001), and boronate affinity chromatography (Brownlee et al., 1980) followed by mass spectrometry. However, the factors listed below have contributed to the lack of sensitivity and selectivity in the methods for identification and quantitation of glycated proteins: (1) the low concentration of glycated proteins; (2) the modification of enzymatic digestion patterns; (3) the low ionization efficiency of glycated peptides, and (4) the lack of software including tools to identify glycation modifications (Priego Capote and Sanchez Capote, 2009). Proteomic analysis of glycated proteins involves three major steps including sample preparation followed by protein separation with one dimensional or two dimensional gel electrophoresis, enzymatic hydrolysis of proteins, chromatographic separation of the resulting peptides followed by MS analysis and finally data processing involving the database search. However, these conventional proteomics protocols have been scarcely used in the analysis of glycated proteins due to two main reasons. The first reason is low concentration of glycated proteins and secondly the information about the preferred glycation sites for each protein could be lost during conventional enzymatic digestion of proteins. For this reason, it becomes mandatory to enrich the glycated proteins by using affinity columns, and liquid chromatography techniques.

Boronic acid chromatography (BAC) involves esterification between boronic acid and 1, 2-cis-diol compounds (possessing two hydroxyl groups of the diol on adjacent carbon atoms) under alkaline conditions which is particularly observed mainly at the glucose moiety in the case of protein glycation. Therefore, BAC is selective for enrichment of non-enzymatically glycated proteins (Li et al., 2001). Identification of glycated proteins can be achieved by phenylboronate affinity chromatography coupled with reversed-phase LC-MS/MS and utilizing data-dependent tandem mass spectrometry with alternating electron transfer dissociation (ETD) and collision induced dissociation (CID) MS/MS (Zhang et al., 2007a). However, by using ETD as the fragmentation mode the glycated peptides can be identified with higher confidence as the fragments containing the labile Amadori
A specific modulation in boronic acid known as methacrylamido phenylboronic acid has been successfully used in SDS-PAGE for analysis of glycated proteins. 1% methacrylamido phenylboronate acrylamide gel electrophoresis (mP-AGE) shows retention of d-gluconolactone modified proteins and the method is highly selective for glycated proteins (Morais et al., 2010).

Mass spectrometric analysis has shown the development of the following methods for the characterization of glycation modification of glycated proteins. A reverse phase liquid chromatography method followed by a neutral loss scan method consists of two segments for the screening of the glycated peptides. First segment involving identification of glycated peptides based on the neutral loss of 162 Da while the second segment involving selection of glycated peptide as parent ion and further fragmentation at higher collision energy to break the peptide bonds which reveals the amino acid sequences and the sites of glycation (Gadgil et al., 2007).

Metz and co-workers proposed several approaches for the characterization of glycated proteins which are primarily based on bottom-up work flows (Zhang et al., 2008a; Zhang et al., 2008b; Zhang et al., 2009; Zhang et al., 2011). Nevertheless, these approaches have been focused on only qualitative analysis. Therefore, for quantitative analysis of glycated proteins a differential labelling of proteins with isotopically labelled sugars (13C-sugars), named glycation isotopic labelling (GIL) has been evolved. Because of the chemoselectivity of GIL, only preferential glycation targets are labelled followed by analysis of these non-enzymatic glycation sites in the human plasma proteome (Priego-Capote et al., 2010).

I.5. Genesis and Organization of Thesis

An increased blood plasma glucose level is the hallmark of diabetes resulting into plasma protein glycation. The involvement of protein glycation in the pathogenesis of diabetic complications has evoked a special medical interest in preventing the glycation of proteins in human. In this regard, it has become equally important in gaining knowledge about plasma protein glycation and its regulation in
vivo. For addressing this question, preliminary studies in determining the factors influencing glycation reaction were performed. Further, albumin constitutes about 50% of plasma proteins and is one of the heavily glycated protein due to its abundance. Therefore any variation in albumin levels may change the stoichiometry of plasma protein glycation. To understand the role of albumin mediated glycation regulation, a systematic and comprehensive plasma proteome analysis of diabetic and non-diabetic subjects were performed. In addition, the differential expression of plasma proteins in non-diabetes, controlled diabetes and poorly controlled diabetes contributing in the development of diabetic complications has been discussed.

**Major objectives of the thesis are as follows**

- To study various factors influencing the glycation reaction *in vitro*
- To study the association of albumin in the regulation of glycation *in vitro* and *in vivo*.
- To study the differential expression of plasma proteins in poorly controlled diabetes

**This thesis is organized and presented in the following manner**

- Chapter I: Introduction
- Chapter II: Factors influencing glycation reaction *in vitro*
- Chapter III: Association of albumin levels with plasma protein glycation and HbA1c in diabetes
- Chapter IV: Proteomic study reveals down regulation of apolipoprotein A1 in plasma of poorly controlled diabetes
- Summary and Future Perspectives
- Bibliography
- Appendix I & II (List of glycation modification and their MS MS annotations)