2.1 BACKGROUND

2.1.1 Pathogenesis and Pathophysiology of Diabetes Mellitus

The main culprit for type I diabetes mellitus is autoimmune destruction of pancreatic β-cells. Evidence of this autoimmune process includes the presence of islet cell antibodies, insulin autoantibodies and antibodies to glutamic acid decarboxylase (Forrest et al., 1991). The factors that initiate the autoimmune destruction of pancreas are unknown; Viruses and chemical toxins have been suggested as initiating agents of IDDM. Animals and in humans the role of viruses, Coxsackie virus B, in the pathogenesis of type I diabetes is very clear. Other environmental factors like dietary changes, temperature, distance from the equator and ethnicity of the population enhance the risk of developing diabetes (Engelgau et al., 1995). Most patients with IDDM present with one or more symptoms of polyuria, polydipsia, excessive hunger, and fatigue or weight loss with elevated levels of glucose and ketones in blood and urine (Langer 1994).

NIDDM, maturity onset diabetes of young, also may be present with classical symptoms but often is asymptomatic. NIDDM is the consequence of a deficiency in insulin action due to abnormalities at the cell surface or with in the cell, a deficiency in insulin secretion or a combination of these processes (Gruppuso et al., 1984). The primary cause of NIDDM is reduction in glucose stimulated insulin secretion. The deficit in insulin reaction results in increased levels of blood glucose and other metabolic disturbances, but not in disturbances of lipolysis severe
enough to produce clinical ketonemia or metabolic acidosis. NIDDM can present with classical diabetic symptoms and signs such as thirst, polyuria, polyphagia, pruritus, and weight loss.

Along with hyperglycemia and abnormalities in serum lipids, diabetes is associated with micro vascular and macro vascular complications which are the major causes of morbidity and death in diabetic subjects (Karjalainen et al., 1988). There is a similar progression of complications in type 1 and type 2 diabetes and there is a high incidence of retinopathy, nephropathy in type 2 diabetes at the time of diagnosis (Pandit et al., 1993). Further, diabetes increases risk of cardiovascular diseases as shown in various studies (Fig. 3 pg.no 39).

Diabetes mellitus accelerates the process of atherosclerosis and increases the risk of heart attack. Cells in the retina and lens of the eye are usually damaged in diabetes (Eizirick et al., 1996). It can destroy the filtration system of the kidney. It kills nerves and with dead nerves, feet lose sensation making them injury prone. Because blood flow is impeded, wounds heal more slowly, and infections get out of hand. Men become impotent due to the damage of nervous and circulatory systems. Congenital defects and still births are associated problems with gestational diabetes (Berelowitz and Eugene, 1996). Most worrying complications of diabetes include loss of vision, heart attack, kidney failure, limb amputation and impotency. Although the "Diabetes Control and Complications Trial" has identified hyper glycaemia as a risk factor for development of diabetic complications, there are a number of equally
tenable hypotheses on the origin of complications (Ronald Kahn et al., 1997).

These hypotheses include advanced glycation end product hypothesis, the aldose reductase hypothesis, oxidative stress, reductive stress, true hypoxia, carbonyl stress, altered lipoprotein metabolism, increased Protein kinase-C activity and altered growth factor or cytokine activities.

2.1.2 Genetic susceptibility:

Type-1 diabetes mellitus occurs frequently in persons of Northern European descent compared to Native Americans, Asians and among other radical groups including blacks (Kyvik et al., 1995). At least one of the genetic susceptibility genes for type-1 diabetes resides in the region that encodes the class II antigens of the MHC on chromosome 6P21 (HLAD) (Bain et al., 1997). The genetic variation in the HLA class II molecule may alter recognition by the T-cell receptor or modify the presentation of antigen causing variation in antigen binding cleft. Thus, class II HLA genes may affect the degree of immune responsiveness to a pancreatic β-cell autoantigen, or a β-cell auto antigen may be present in a manner that promotes an abnormal immunological reaction (Reed et al., 1997).

2.1.3 Autoimmunity:

This disease, in fact, results from a chronic autoimmune attack of β-cells that is associated with increased expression of class 1 MHC molecule and aberrant expression of class II MHC molecules on the
Chapter-II  

**Background and Review of Literature**

β-cells (Laufer et al., 1993). The aberrant expression is mediated in part by locally produced cytokines (IFN-γ) derived from activated T cells. It produces the development of diabetes in mouse model (Rothe et al., 1997).

2.1.4 Environmental factors:

Epidemiological studies suggest that the action of viruses may cause type-1 diabetes. Seasonal trends that often correspond to the prevalence of common viral infections have long been noted in the diagnosis of new causes, coxsackie virus of group B cause diabetes and pancreatic diseases (Verge et al., 1996). The viruses cause mild β-cell injury which is followed by an autoimmune reaction by virally altered β-cells with HLA linked susceptibility.

2.1.5 Pathogenesis of Type-II diabetes mellitus:

Epidemiological studies indicate that type-2 diabetes appears to result from a collection of multiple genetic defects or problem of polymorphisms, each contributing its own predisposing risk and modified by environmental factors. A deranged β-cell secretion of insulin and a decreased response of peripheral tissues responding to insulin (insulin resistance) are the two major metabolic defects that characterize type-2 diabetes.

2.1.6 Deranged β-cell secretion of insulin:

Risk for developing type-2 diabetes, a modest hyperinsulinemia, may be attributed to β-cell hyper responsiveness to physiological elevations in blood glucose. Observations suggest that derangements in β-
cells responses to hyperglycemia and a mild to moderate deficiency of insulin develops in type-2 diabetes. According to one view, all the somatic cells are genetically vulnerable to injury, leading to accelerated cell turnover and premature aging, and ultimately to a modest reduction in $\beta$-cell mass (Ronald Kahn et al., 1997)(Fig. 4pg.no 40).

2.1.7 Insulin resistance:

In type-2 diabetes, there may be a decrease in the number of insulin receptors, and post receptor signaling by insulin is impaired. In this condition that reduces synthesis and translocation of glucose transporters(GLUTs) in muscle and fat cells underlies the insulin resistance noted in obesity. The mobility of circulating insulin properly directs the disposition of glucose and a more persistent hyperglycemia. Therefore, more prolonged stimulation of pancreatic $\beta$-cell for the production of insulin is required (Ronald Kahn, 1997).

2.1.8 Obesity:

Life style plays a clear role as is evident from the study of obesity. Abdominal obesity and insulin resistance could be coincidental expression of third unknown factor; the possibility whether they are causally related must be considered (Groop, 1997).

2.1.9 Management of diabetes mellitus:

Type 2 diabetes is usually treated by a combination of diet, exercise and life style changes, or pharmacological agents (e.g., oral antidiabetic agents and insulin).
2.1.9.1 Diet and life style changes:

Medical nutrition therapy is an essential component of diabetes management; unfortunately, patient adherence to nutrition principles is one of the most challenging aspects of diabetes care. A goal of medical nutrition therapy is to achieve and maintain blood glucose concentrations as close to normal as possible by balancing food intake with antidiabetic drug therapy and physical activity levels. Not more than 30% of the total daily caloric intake should come from fats; 10% to 20% from protein, and the balance of daily calories from carbohydrates. Exercise improves insulin sensitivity and glycemic control, especially in patients with mild diabetes or a high degree of insulin resistance (ADA, 2007).

2.1.9.2 Sulphonylureas:

Sulphonylureas are a class of compounds containing sulphonamide drug. These sulphonylureas lower the blood glucose in vivo and in vitro by increasing the plasma insulin levels. These chemicals stimulate the secretion of insulin from β-cells. The receptors of sulphonylurea are present in cardiac muscle cells, smooth muscle cells, liver and adipose tissue. There are several side effects mediated by the use of sulphonylureas. They are chronic renal failure, hepatic and cardiovascular diseases (Zimmerman, 1997).

2.1.9.3 Biguanides:

Biguanides are derivatives of guanide especially phenformin, metformin and buformin. Recently metformin gained popularity in diabetic
patients because of its variations in the chemical structure. These biguanides enhances the utilization of glucose in peripheral tissues and increases the gluconeogenesis in liver. Metformin treatment does not stimulate insulin secretion, but it improves insulin mediated glucose uptake.

Lipid peroxidation and lipolysis are reduced by metformin. The side effects of the usage of metformin are anorexia, nausea, diarrhea and unpleasant metabolic taste (Holman and Turner, 1991).

2.1.9.4 α-glycosidase inhibitors:

α-glycosidase inhibitors are a group of compounds which inhibit the rate of breakdown of oligosaccharides and polysaccharides. This delays the absorption of glucose. These drugs can be used in combination with others to decrease the blood glucose levels (Hanefeld et al., 1991).

2.1.9.5 Thiazolidinediones:

These are oral hypo glycemic agents like cigilitazone, troglitazone and rosiglitazone are antidiabetic drugs which are currently available for clinical use in the treatment of diabetes. The mechanism of the action of these drugs is not completely understood. The usage of these drugs leads to the alteration of the enzyme activities in liver mainly affecting the transaminases.
2.1.9.6 Oral hypoglycemic agents:

The present treatment of diabetes is focused on controlling and lowering blood glucose. The mechanisms to decrease blood glucose in western medicines are i) to stimulate β-cells of pancreatic islet to release insulin; ii) to resist the hormones which rise blood glucose; iii) to increase the number or rise the appetency and sensitivity of insulin receptor site to insulin; iv) to stimulate the beta cell stimulators act at the level of the pancreatic beta cells to stimulate insulin release. They require the presence of functioning beta cells, and used only in the treatment of type 2 diabetes, and have the potential for producing hypoglycemia. The sulfonylureas reduce blood glucose by stimulating the release of insulin from beta cells in the pancreas and increasing the sensitivity of peripheral tissues to decrease the hydrolysis of glycogen; v) to enhance the use of glucose in tissue and organ; vi) to clear away free radicals, resist lipid peroxidation and correct the metabolic disorder of lipid and protein; and vii) to improve microcirculation in the body (Li et al., 2002).

Metformin, the only currently available biguanide, inhibits hepatic glucose production and increases the sensitivity of peripheral tissues to the actions of insulin. Secondary benefits of metformin therapy include weight loss and improved lipid profiles. Unlike the sulfonylureas, whose primary action is to increase insulin secretion, metformin exerts its beneficial effects on glycemic control through decreased hepatic glucose production (main effect) and increased peripheral use of glucose. This
medication does not stimulate insulin secretion; therefore, it does not produce hypoglycemia.

The α-glycosidase inhibitors block the action of the brush border enzymes in the small intestine that break down complex carbohydrates. By delaying the breakdown of complex carbohydrates, the α-glycosidase inhibitors delay the absorption of carbohydrates from the gut and blunt the postprandial increase in plasma glucose and insulin levels. The postprandial hyperglycemia probably accounts for sustained increases in HbA1c levels.

The thiazolidinediones (TZDs), or glitazones, are the only class of drugs that directly target insulin resistance, a fundamental defect in the pathophysiology of type 2 diabetes. The TZDs improve glycemic control by increasing insulin sensitivity in the insulin-responsive tissues, liver, skeletal muscle, and fat allowing the tissues to respond to endogenous insulin more efficiently without increased output from already dysfunctional beta cells.

### 2.1.9.7 Insulin therapy:

Insulin is an important hormone needed by the human body to utilize carbohydrates, protein, and fats. However, in type 1 diabetes the pancreas does not produce insulin, and replacement therapy is required with exogenous insulin. Type 2 diabetics, on the other hand, have a problem with either the secretion of insulin or have become insulin-resistant; thus, the common name for the condition is non insulin-resistant.
dependent diabetes mellitus. Insulin injections are a necessary daily component of therapy for type 1 diabetics. Insulin injections however, are not always necessary for treatment and control of diabetes in type 2 diabetics (Buse, 1999).

2.1.9.8 Disadvantages of insulin therapy:

The major disadvantage associated with insulin therapy is incidence of insulin allergy and insulin resistance. Insulin injection causes a localized loss of subcutaneous fat in the area of injection. Insulin therapy is also associated with weight gain. The maintenance of blood glucose requires the addition of OHA’s (Oral Hypoglycemic Agent) along with the insulin. With the progress of the disease exogenous intake of insulin suppresses the secretion of insulin and leads to the loss of β-cells. Keeping the above points in view, WHO declared that the diabetes is one of the priority areas of research with a slogan “Prevent and Cure Diabetes”.

2.1.9.9 Indigenous treatment for diabetes mellitus:

Practice of healing is known as medicine which is an important branch of science. From times immemorial human beings (animals and birds also) self medicate themselves with natural sources of animal and plant origin to get rid of their illness, use of herbs has been practiced for centuries in all parts of the world in various systems of medicine like Ayurveda, Siddha, Unani and Naturopathy etc. A reference to a number of herbal remedies has been made in Vedas (Rigveda and Atharvanaveda).
2.1.9.10 Prevalence of Diabetes in India:

A national survey of diabetes conducted in six major cities in India in the year 2000 has shown that the prevalence of diabetes in urban Indian adults was 12.1% (Ramachandran et al., 2001). The onset of diabetes among Indians is about a decade earlier than their western counterparts and this has been noted in Asian Indians in several studies (Ramaiya et al., 1990).

In the national survey 54.1% of diabetes developed it in the most productive years of their lives i.e. before the age of 50 years and they also had a higher risk of developing chronic complications of diabetes (Ramaiya et al., 1990; Ramachandran et al., 1992). The prevalence of Type 2 diabetes is 4-6 times higher in the urban areas as compared to rural areas. The prevalence of impaired glucose tolerance (IGT) in the rural population is also high at 7-8%, which indicates presence of a genetic basis for Type 2 diabetes in ethnic Indian population (Viswanathan et al., 1996).

2.1.10 Complications of diabetes mellitus:

The complications of diabetes mellitus are a heterogeneous group of clinical disorders which can affect the vascular system, kidney, eye, nervous system and other tissues.

2.1.10.1 Microvascular complications:

Microvascular complications include neuropathy (nerve damage), nephropathy (kidney disease) and vision disorders (eg: retinopathy, glaucoma, cataract and corneal disease).
2.1.10.2 Macrovascular complications:

Cardiovascular disease is the primary cause of early mortality in patients with type 2 diabetes. Coronary heart disease, hypertension, stroke and peripheral vascular disease occur with high frequency in diabetics due to altered lipid profile.

2.1.10.3 Retinopathy:

Diabetic retinopathy (DR) occurs in about 95% of patients with type 1 diabetes mellitus (DM) and in 60% of type 2 DM patients. DR is the most common cause of blindness and characterized by increased proliferation of blood vessels, vascular occlusion, angiogenesis, microaneurysms, haemorrhages and infarction affecting the retina of the eye and hard exudates are described as 'background retinopathy' or preferably nonproliferative retinopathy. The nonproliferative retinopathy occurs near the maculae and causes macular edema. Macular edema occurs when leakage of fluid from abnormal vessels near the macular disrupts the light path to the macule and results in the loss of visual acuity (Nathan et al., 1986). These changes are accompanied by thickening of the capillary basement membrane, increased permeability of capillaries, loss of pericytes and increased endothelial cell turnover and death (Krolewski et al., 1988). Other visual damage caused or facilitated by diabetes includes cataract, keratitis, and optic nerve damage.
2.1.10.4 Nephropathy:

Diabetic nephropathy is characterized by a thickening of the basement membrane, expansion of the mesangium, reduced filtration, albuminuria and ultimately renal failure (Mauer and Drummonder., 2002). The renal lesions underlying renal dysfunction differ in type 1 and type 2 diabetes, although the clinical manifestations of diabetic nephropathy, proteinuria, decreased glomerular filtration rate and increasing blood pressure are similar. Indeed, in type 1 diabetes, although also tubular, interstitial and arteriolar lesions are present, the most important structural changes involve the glomerulus, while several type 2 diabetic patients, despite the presence of microalbuminuria or proteinuria (30 to 300 mg of albumin per 24 hours), have normal glomerular structure with or without tubulo-interstitial and arteriolar abnormalities, which may occur as early as five years after the onset of diabetes (Viberti and Keen, 1984). This stage of incipient nephropathy may be more likely in patients with glomerular hyperfiltration. Overt diabetic nephropathy is clinically characterized by proteinuria, nephritic syndrome development and the falling of glomerular filtration rate resulting in end stage renal disease (Mogensen, 1997).

2.1.10.5 Neuropathy:

Diabetic neuropathy is characterized by segmental demyelination and axonal degeneration of peripheral neurons, together with functional abnormalities such as reduced nerve conduction and blood flow. Diabetic neuropathy may be present clinically as pain or numbness of limbs or as impotence in men. There is increased glycation of myelin in diabetes. The
progression of neuropathy is dependent on the degree of glycemic control in both Type 1 and Type 2 diabetes. A peripheral symmetric sensorimotor neuropathy is the most common form of diabetic neuropathy, whose other forms include cranial and peripheral motor neuropathies and autonomic neuropathy. Although neuropathy is more common with a longer duration of diabetes (Said et al., 1992), the principal risk posed by peripheral neuropathy is of foot trauma and diabetic ulcer. A minority of patients have painful peripheral neuropathy with lancinating or burning dysesthesia, severe enough for some to be associated with depression and anorexia (Ellenberg, 1974). Risk factors for diabetic neuropathy are duration of diabetes, age, cigarette smoking, hypertension, height and hyperlipidemia.

2.1.10.6 Embryopathy:

Diabetic mothers with poor glycemic control are prone to embryopathy, where the newborn has an increased frequency of congenital malformations. The precise mechanism underlying embryopathy in diabetes is unknown, but a reduction in congenital malformations is seen in pregnancies where the hyperglycemia is well controlled (Mills et al., 1988). Embryopathy may arise because of glycation of DNA and histones by reactive intracellular sugars and indeed increased AGEs have been detected on his tones isolated from diabetic rats (Gugliucci and Bendayal, 1995). Glycation and AGE formation on DNA and histones could cause errors in replication and transcription thereby promoting mutations responsible for embryopathy. However, the
cause of diabetic embryopathy is likely to be multifactorial as elevated concentrations of ketone bodies and branched chain amino acids have also been implicated in its pathogenesis (Eriksson et al., 1998).

2.1.10.7 Diabetic foot:

Foot ulceration is a prominent cause of diabetes mellitus morbidity and mortality in developing countries. A problem in diabetic patient is the development of ulcers in the feet and lower extremities and is attributed primarily to abnormal pressure distribution, secondary to diabetic neuropathy. Diabetic motor neuropathy is expressed as the loss of function and the contracture of the intrinsic muscles of the foot, leading to the classic claw toe deformity. This deformity predisposes the foot to ulcerations on the dorsum or tip of the toes (Kim et al., 2008). The risk factors/precipitants of foot ulceration include neuropathy, vasculopathy, spontaneous blisters, walking unshod, and wearing inadequate shoes. Prominent hematologic abnormalities include anemia and leucocytosis.

2.1.10.8 Cardio vascular diseases:

Cardio vascular disease is generally similar in patients with type 1 or type 2 diabetes and patients without diabetes. Mortality from first or subsequent myocardial infarctions is higher in diabetic than non diabetic patients (Singer et al., 1989). Patients with NIDDM and impaired glucose tolerance are commonly obese and have hypertension and dyslipidemia (increased serum triglyceride and decreased HDL cholesterol levels). However, independently of these variables, diabetes remains a major risk
factor for coronary artery disease (Singer et al., 1992). The levels of chronic glycaemia, as determined by measurements of glycosylated hemoglobin, may also be an independent risk factor for coronary artery disease (Singer et al., 1992).

2.1.10.9 Hypertension and stroke:

The diagnosis of type 2 DM is often made 4 to 7 years after the disease process has begun, when most patients already have an increased risk of macrovascular processes. Despite this, 20% to 25% of patients with DM do not develop macrovascular complications (Koda-Kimble and Carlisie, 1995). However, people with diabetes have 2-8-fold risk for cardiovascular mortality than people without diabetes. Diabetic patients have about twice the prevalence of hypertension and about twice the incidence of stroke compared to non-diabetic patients (Zimmet and Alberti, 1998). An increased prevalence of hypertension and concurrent lipid abnormalities (i.e., abnormally decreased high density lipoprotein, elevated low-density lipoproteins, and elevated triglycerides) may be responsible for macrovascular complications in patients with DM. Hyperglycemia and hyperinsulinemia also have been implicated as contributors to macrovascular complications, although it is difficult to determine the extent of their contribution (Savage, 1996). Reduction of the degree and duration of hyperglycemic episodes through aggressive control of blood glucose can lower the risk of macrovascular complications, although this has not been confirmed.
2.1.10.10 Diabetic Ketoacidosis:

Diabetic Ketoacidosis (DKA) develops due to either an absolute or a relative absence of insulin. An absolute insulin deficiency is the major precipitant for those patients presenting in DKA who have new onset type I diabetes. It is estimated that 10-20% of patients with new onset of diabetes will present in DKA as their initial presentation. Another major cause of absolute insulin deficiency is omission of normal insulin in a patient with known type I diabetes (Balasubramanyam et al., 1999). Myocardial infarction should always be considered in the list of precipitating factors of DKA, particularly in older patients, as the condition associated with elevations of epinephrine, which may stimulate a pathologic process that results in DKA. Diabetic Ketoacidosis is secondary to increased serum levels of Ketoacids in an individual with type I diabetes mellitus.

2.2 REVIEW OF LITERATURE:

2.2.1 Diabetic nephropathy:

Diabetic nephropathy is a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli (Fig. 5 pg. no 41). It is characterized by nephrotic syndrome and diffuse glomerulosclerosis. It is due to longstanding diabetes mellitus, and is a prime indication for dialysis in many Western countries.
2.2.2 Progression of chronic renal failure:

There are several conditions and diseases that can lead to chronic kidney disease (CKD). Like most other organs in the human body, the kidney is prone to a gradual loss of function over the decades. This physiological phenomenon is clearly demonstrated by the gradual fall in glomerular filtration rate with increasing age (Levi et al., 2003). Fortunately, this small decrease in renal function is usually of no clinical significance. However, in patients with a renal disorder, the rate of renal function loss can be significantly enhanced, even if the primary insult or underlying disease activity has already abated. This will result in several metabolic disturbances, and eventually, for many patients, in end-stage renal failure. The course of renal function loss can be described as the reciprocal of serum creatinine level against time, resulting in a straight line in the majority of patients. Interestingly, the rate of renal function loss shows a remarkable inter-individual variability between patients, even if they have a similar underlying disorder. This suggests that progression of renal function loss appears to be largely independent of the type of underlying renal disease, but that other factors are involved. It is obvious that the prevention of progressive renal failure is of paramount importance to the patient and also to reduce the high costs for the treatment of these patients. Unfortunately, we are still not capable to prevent the onset of many types of renal diseases. However, numerous observations have made it clear that progression of renal function loss is largely dependent upon secondary factors (De Zeeuw et al., 1990; Remuzzi, Benigni, 1997).
Systemic and glomerular hypertension, proteinuria and hyperlipidemia are assumed to be the most common mediators in the final common pathway of progressive renal damage (de Zeeuw et al., 1990). These common risk factors are often present simultaneously and mutual interaction of these risk factors appears to accelerate progressive renal damage.

Hypertension and diabetes are just two of the most common causes. When we talk about hypertension is blood pressure. Blood pressure is determined by the force of blood being pumped from the heart, and force of blood against the walls of the arteries. When uncontrolled, blood pressure can be life threatening. Blood pressure that is high can make the heart work too hard, harden the walls of arteries, and can lead to a stroke or brain hemorrhage. It can also cause the kidneys to function poorly or not at all. A blood pressure reading of 140/90 mmHg or higher is considered high. Normal blood pressure is less than 120/80 mmHg.

More than 65 million American adults have high blood pressure according to the National Institute of Health (NIH). The disease is more common among African Americans, and can lead to worse complications. Therefore, African Americans are at greater risk, not only to develop the disease, but also to suffer its consequences. African Americans are more likely to develop the type of hypertension that can be controlled by salt restriction. It is especially important for African Americans to undergo screening tests for hypertension and seek treatment early.
Over time, uncontrolled high blood pressure can damage the blood vessels and nephrons (functional units of the kidneys) in the kidneys. This causes the nephrons to stop doing their job of filtering out wastes, sodium and excess fluids from the blood. With no place to go, the extra fluids and sodium linger in the bloodstream, putting extra pressure on the walls of the blood vessels, and raising the blood pressure. This extra pressure damages the kidneys even further. Reports from US suggest that most of the kidney disorders were developed due to the above said problems (Fig. 6 pg.no 42). But in addition to that some of the people develop kidney failure due to the consumption of pollutant water.

Diabetic nephropathy represents a major complication in patients with either type I or type II diabetes. The contribution of a 287bp insertion/deletion (I/D) polymorphism of the gene encoding angiotensin-I converting enzyme (ACE) has been investigated and the deletion type is documented to be a risk factor in the development of this disease.

**2.2.3 Increased prevalence of diabetic nephropathy in South Asians:**

Racial differences in the prevalence of diabetic renal disease have been reported. Asian subjects have significantly (p<0.01) higher prevalence (52.6%) of diabetic end stage renal disease (ESRD) when compared with the Caucasians (36.2%) migrant Asian (Young et al., 2003). Indians had 40 times greater risk of developing ESRD when compared with the Caucasians (Chandie Shaw et al., 2002). The prevalence of diabetic nephropathy in type 2 diabetic subjects in India
was reported to be 5-9% (Acharya and Chawla, 1978; Chugh et al., 1989; John et al., 1991). Patients with diabetic nephropathy, especially with type 2 diabetes, have a high cardiovascular risk. The risk for cardiovascular disease (CVD) was 3 fold higher in South Indian NIDDM subjects with nephropathy when compared with their non-nephropathic counterparts (Viswanathan et al., 1998). Thus, in type 2 diabetes, many patients may not reach end stage renal disease due to premature death from CVD.

2.2.4 Abnormal mechanisms involved in diabetic nephropathy:

The exact cause of diabetic nephropathy is unknown, but various mechanisms postulated are hyperglycemia (causing hyper filtration and renal injury), age and activation of cytokines. Hyperglycemia increases the expression of transforming growth factor beta (TGFβ) in the glomeruli and of matrix proteins specifically stimulated by this cytokine. TGFβ may contribute to both the cellular hypertrophy and enhanced collagen synthesis observed in diabetic nephropathy. In a study from southern India (Vijay et al., 2005), it was shown that TGF-β1 levels are elevated in type 2 diabetic subjects. Treatment with insulin and angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) appears to decrease the levels of TGF-β1. Hyperglycemia also may activate protein kinase C, which may contribute to renal disease and other vascular complications in diabetes. In addition to the renal hemodynamic alterations namely decreased glomerular filtration rate and renal plasma flow, patients with overt diabetic nephropathy (dipstick positive proteinuria and decreasing GFR) develop systemic hypertension. Hypertension is an adverse factor in all progressive renal diseases and seems especially so
in diabetic nephropathy. The deleterious effects of hypertension are
directed at the macro and microvasculature.

2.2.5 Pathophysiology of diabetic nephropathy:

The pathogenesis of diabetic nephropathy is multifactorial and
genetic susceptibility has been proposed to be an important factor in the
development and progression of diabetic nephropathy. Two major
causative factors have been implicated in the development of diabetic
nephropathy: metabolic and hemodynamic. Three major histologic
changes occur in the glomerulur diabetic nephropathy (1) mesangial
expansion is directly induced by hyperglycemia, perhaps via increased
matrix production or glycosylation of matrix proteins, (2) Thickening of
glomerular basement membrane (3) Intraglomerular hypertension causing
glomerular sclerosis. These different histologic patterns appear to have
similar prognostic significance. Diabetes produces qualitative and
quantitative changes in the composition of the capillary basement
membrane and this altered material undergoes accelerated glycosylation
and further rearrangement to form advanced glycosylation end-products
(AGE), which stimulate protein synthesis (Doi et al.,1992), further
decrease in degradability of the basement membrane (Brownlee et al.,
1988), increase its permeability (Esposito et al.,1992) and causes
endothelial dysfunction (Bucala et al.,1991).Impaired endothelial function
measured as elevatedendothelin-1 levels and abnormal flow mediated
dilatation has been demonstrated in south Indian type 2diabetic subjects
(Mamatha et al.,2004; Vijay et al.,2004).
2.2.6 Factors controlling diabetic nephropathy:

2.2.6.1 Genetical:

Familial factors may play a role in the development of diabetic nephropathy. Certain ethnic groups, particularly American blacks, Hispanics, and Native Americans, may be particularly predisposed to renal involvement as a complication of diabetes. The (Vijay et al., 1999) conducted a study to determine familial aggregation of diabetic nephropathy in South Indian type 2 diabetic subjects. It was found that proteinuria was present in 50% and microalbuminuria in 26.7% of the diabetic siblings of probands with diabetic nephropathy. In contrast, the prevalence of proteinuria and microalbuminuria among diabetic siblings of probands with normoalbuminuria was 0% and 3.3% respectively (P=0.057 for microalbuminuria). Some evidence has suggested that polymorphism in the gene for the angiotensin-converting enzyme (ACE) contributes in either predisposing to nephropathy or accelerating its course. In a study from south India (Vijay et al., 2001), it was shown that a positive association exists between the ‘D’ allele (ID and DD genotype) of the ACE polymorphism and proteinuria in South Indian type 2 diabetic patients. However, definitive genetic markers have yet to be identified. In summary, hyperglycemia sets in motion a number of hemodynamic and metabolic abnormalities that eventuate in the typical histologic and clinical picture that is seen in patients with diabetic nephropathy.

2.2.6.2 Genetic polymorphisms:

Recent advancements in molecular genetic techniques have provided new insights in the role of genetic variability in renal disorders
with respect to the likelihood to develop renal disease, the course of renal
disease and the benefit of renoprotective therapy. Much effort has been
put in discerning the role of genetic polymorphisms. Mutations in the
human genome occur frequently. Their consequences can be variable. A
first possibility is that the affected offspring is spontaneously aborted or
becomes burdened with clear clinical signs of disease. By this mechanism
the mutation cannot easily be passed on to a next generation. This is
called selection pressure. However, if the mutation does not have
deleterious consequences, offspring can survive and appear relatively
healthy. In some cases subjects develop disease but also have
advantages, for example patients with sickle cell anemia who are less
prone to acquire malaria

2.2.6.3 Genetic polymorphisms as a new clue for disease susceptibility

It is hypothesized that the complex interaction between multiple
environmental factors and inherited genetic polymorphisms could result in
the susceptibility to certain diseases or to modify the course of diseases,
by having each a small, but additive impact. It was recently suggested
that genetic polymorphisms can affect the susceptibility to fairly common
diseases as hypertension, diabetes mellitus, osteoporosis and Alzheimer
disease (Yoshida et al., 1996). However, the path to dissect the impact of
genetic polymorphisms is not an easy one. As each polymorphism
appears to have only a small impact on the phenotype, identification of
such susceptibility genes is far more difficult compared to the identification
of major chromosomal abnormalities or single gene mutations. This is
because even the presence of several susceptibility alleles at multiple loci does not necessarily lead to overt clinical symptoms, postulated to be caused by a subtle difference in exposure to environmental influences (Holtzman and Marteau, 2000).

2.2.6.4 Genetic polymorphisms and renal disease

Careful characterization of clinical phenotypes and detection of genetic markers for the susceptibility to acquire renal diseases or modify its course has been difficult and cumbersome. However, several genetic variabilities have been linked to renal disease. Gene variation of the complement system has been identified with the occurrence of IgA nephropathy (Wyatt et al., 1991).

2.2.6.5 Angiotensin-converting enzyme insertion/deletion(I/D) polymorphism:

The renin-angiotensin aldosterone system (RAAS) has a key role in both cardiovascular and renal pathophysiology (Dzau, 1994; Wolf, 1998). Angiotensin II (angII), the most important biological active product, is synthesized via a pathway that involves several precursor peptides and enzymes, some of them regulated by individual genes. The main known action of angII is its potency to constrict vascular smooth muscle cells and to stimulate fluid and sodium retention, by directly acting on tubular cells and through stimulating aldosterone release. However, more recently other potential actions of angII have been elucidated. Several studies have revealed that angII in vitro promotes vascular smooth muscle,
glomerular mesangial and renal tubular cell growth (Dzau, 1994; Wolf, 1998). AngII also appears to be involved in the accumulation of collagen by both activating collagen synthesis and inhibition of its degradation (Yoshida et al., 1996). Therefore, it is clear that genetic polymorphisms of the RAAS have gained interest in the search for genetic factors that might influence the progression of chronic renal failure and the response to treatment to renoprotective regimens.

Angiotensin I converting enzyme (ACE) is a zinc metallopeptidase widely distributed on the surface of endothelial and epithelial cells. By stimulation of renin, angiotensinogen is converted to angiotensin I. ACE then converts angiotensin I to angiotensin II, the main active product of the RAAS. The human ACE gene is located on chromosome 17 and includes 26 exons. The coding sequence codes for a 1306 amino-acid protein, including a signal peptide. The gene product, ACE, is composed of 2 homologous domains with 2 active sites.

2.2.6.6 Association with ACE polymorphism:

Diabetic nephropathy, usually proceeded by hypertension and persistent albuminuria, eventually develops in 30 to 50% of patients with insulin-dependent (IDDM) or non-insulin-dependent (NIDDM) diabetes mellitus and is one of the leading causes of end-stage renal failure in most industrialized countries. Various drugs such as the α-blockers, β-blockers, calcium channel blockers, ACEI and ARB are available to target hypertension. But the key to initiate treatment is not to get everyone to
one particular class of drug, but to treat effectively with the best drug for individual patients. Even though α-blockers, β-blockers and calcium channel blockers remain important anti-hypertensive agents in diabetic patients, they should be considered as second or third line drugs in combination with ACEI and ARB. On the basis of the finding that both diabetic nephropathy (Seaquist et al., 1989) and hypertension (Krolewski et al., 1988) tend to cluster in families, it is anticipated that genetic markers can be identified that would allow earlier detection of this devastating complication in diabetic individuals.

The renin-angiotensin system plays a central role in BP regulation and renal function not only as a key regulator of sodium homeostasis, but also as a modulator of vascular tone and possibly vascular structure. These effects are mediated primarily by angiotensin II, which is liberated from angiotensinogen by the sequential action of renin and angiotensin-converting enzyme (ACE). The importance of the renin-angiotensin system in the development of diabetic nephropathy is supported by several studies, indicating a beneficial effect of inhibition of this system on the development and progression of this complication (Lewis et al., 1993; Parving et al., 1995). Genes coding for the components of the renin-angiotensin system therefore are prime candidate genes for a possible role in the development or progression of diabetic nephropathy.

Recent studies have identified a variant of the angiotensin converting enzyme gene (ACE) involving an intronic deletion (D) of a
289-bp Alu sequence, which has been associated with increased plasma and tissue activity of this enzyme (Rigat et al., 1990; Tiret et al., 1992). This genetic variant has been implicated as a risk factor for left ventricular hypertrophy myocardial infarction (Cambien et al., 1992), and stroke (Markus et al., 1995), but these findings are far from conclusive (Samani et al., 1996). Marreet al. (1994) reported that in patients with IDDM the presence of the ACE-D variant was associated with an odds ratio (or) of 3.88 for developing diabetic nephropathy. Since the appearance of this study, numerous investigators have addressed this issue both in patients with IDDM and NIDDM with inconsistent results. The primary objective of this systematic review, therefore, was to examine the reported association between the ACE-D variant and diabetic nephropathy in an attempt to explain the controversial results in the published literature. This topic is well suited for a meta-analytic approach because all studies postulate the same relationship between nephropathy and the ACE-D variant. Also, all studies have investigated this hypothesis using a similar strategy of comparing allelic or genotype frequencies in nephropathic patients to those in non-nephropathic control subjects.

2.3 Significance of the study:

Obesity is the main cause for several life threatening diseases like diabetes. Particularly in countries like India, where various forms of people live with a unity in diversity. Particularly some communities were more susceptible to diseases like diabetes due to their life style and habituates. In Andhra Pradesh, specifically the vysya community
(Settiyar) is more prone towards obesity due to their lifestyle. People belonging to this community are frequently suffering from diabetes and renal failures. In general most of the studies were conducted to know the ethnic differences between the countries. But studies related to the exact evidence of relation between the community and the diabetic nephropathy is not known. Hence it is very much essential to find out the reasons for the loss of renal system with in the settiyar community.

The whole phenomenon of renal failure was markedly analyzed through ACE, where the expression is differentiated under different conditions. Studies related to ACE polymorphism are also abundant in some other disorders. Here the use of this context was taken to analyze the ACE polymorphism under type 2 diabetes mellitus mediated renal failures. From this background the study was started in the Nellore and Prakasam districts of Andhra Pradesh, which is geographically southern part of the India near to the Bay of Bengal (Fig.7 pg.no 43).

Questionnaires were prepared to identify the samples in and around the selected areas. The families were identified with the present problem and further analysis was conducted with the following aims and objectives:

2.4  **Aim and objectives of the present study:**

2.4.1  **Aim:**

The aim of present research work is to evaluate the prevalence of diabetic nephropathy in the selected community (settiyar) based on their selectable markers.
2.4.2. Objectives:

Objectives of the present study are:

1. To identify selected community people in the Nellore and Prakasam districts with present clinical manifestations (selection was done through questionnaire method).

2. To select known diabetic (KD) subjects and newly diagnosed diabetic (NDD) subjects from the settiyar community.

3. To examine blood and urine parameters for confirmation of diabetes.

4. To assess of renal risk in the above mentioned patients by preliminary analysis of urine.

5. To evaluate hematological parameters viz. neutrophil, basophil, acidophil, leukocyte, total WBC, Hb level etc. in the selected subjects.

6. To evaluate of biochemical parameters viz. Total protein, Glucose, Lipid profiles, SGOT, SGPT etc.

7. To evaluate glomerular and tubular markers viz. $\alpha$-1 microglobulin, $\beta$-2 microglobulin, $\alpha$-1 antitrypsin, IgG and ACE etc.

8. To study association of insertion and deletion II/DD polymorphism in ACE gene 1 in evaluated patients.
Table 1: Top ten countries for estimated number of adults with diabetes, 1995 and 2025 (Hilary King et al., 1998)

<table>
<thead>
<tr>
<th>Rank</th>
<th>Country</th>
<th>1995 (Millions)</th>
<th>Rank</th>
<th>Country</th>
<th>2025 (Millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>19.4</td>
<td>1</td>
<td>India</td>
<td>57.2</td>
</tr>
<tr>
<td>2</td>
<td>China</td>
<td>16.0</td>
<td>2</td>
<td>China</td>
<td>37.6</td>
</tr>
<tr>
<td>3</td>
<td>USA</td>
<td>13.9</td>
<td>3</td>
<td>USA</td>
<td>21.9</td>
</tr>
<tr>
<td>4</td>
<td>Russian federation</td>
<td>8.9</td>
<td>4</td>
<td>Pakistan</td>
<td>14.5</td>
</tr>
<tr>
<td>5</td>
<td>Japan</td>
<td>6.3</td>
<td>5</td>
<td>Indonesia</td>
<td>12.4</td>
</tr>
<tr>
<td>6</td>
<td>Brazil</td>
<td>4.9</td>
<td>6</td>
<td>Russian federation</td>
<td>12.2</td>
</tr>
<tr>
<td>7</td>
<td>Indonesia</td>
<td>4.5</td>
<td>7</td>
<td>Mexico</td>
<td>11.7</td>
</tr>
<tr>
<td>8</td>
<td>Pakistan</td>
<td>4.3</td>
<td>8</td>
<td>Brazil</td>
<td>11.6</td>
</tr>
<tr>
<td>9</td>
<td>Mexico</td>
<td>3.8</td>
<td>9</td>
<td>Egypt</td>
<td>8.8</td>
</tr>
<tr>
<td>10</td>
<td>Ukraine</td>
<td>3.6</td>
<td>10</td>
<td>Japan</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>All other countries</td>
<td>49.7</td>
<td></td>
<td></td>
<td>103.6</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>135.3</td>
<td></td>
<td></td>
<td>300.0</td>
</tr>
</tbody>
</table>
Table 2: Prevalence of diabetes in developed and developing countries (Hilary King et al., 1998)

<table>
<thead>
<tr>
<th>Year</th>
<th>World %</th>
<th>Developed countries %</th>
<th>Developing countries %</th>
<th>India %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>4.0</td>
<td>5.9</td>
<td>3.3</td>
<td>3.8</td>
</tr>
<tr>
<td>2000</td>
<td>4.2</td>
<td>6.2</td>
<td>3.5</td>
<td>4.0</td>
</tr>
<tr>
<td>2025</td>
<td>5.4</td>
<td>7.6</td>
<td>4.9</td>
<td>6.0</td>
</tr>
</tbody>
</table>
Fig. 1: Estimated prevalence of diabetes mellitus of adults in 2030 as compared with data from the year 2000. (Diabetes Care, Vol.27, 2004; 1047-1053).
Fig. 2: Global projections for the diabetes epidemic: 2000-2030 (Diabetes Atlas, 2005).
Fig. 3: Path ways representing the occurrence of type-1 and 2 diabetes (Mohit Josh, 2011).
Fig. 4: Mechanism of blood glucose elevated in type 2 diabetes mellitus
(Mohit Josh, 2011)
Fig. 5: Diagrammatic representation of diabetic nephropathy
(Taken from http://www.ncbi.nlm.nih.gov)
Fig. 6: Annual data of US showing the causes of kidney failures (United States Renal Data System.USRDS 2007 Annual Data Report)
Chapter-II  

Background and Review of Literature

Fig. 7: Geographical region of Nellore and Prakasam districts in Andhra Pradesh, places where the samples were collected.  
(Taken from http://en.wikipedia.org)