Chapter 1

Introduction
1.1 DIABETES

Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose level. The body is unable to metabolize the glucose formed either because of lack of insulin or because the cells are unable to respond to the insulin produced. It is an insidious disease with no clinical symptom in the pre-diabetic stage and only when the disease progress to overt stage, it is characterized by hyperglycemia. The foremost symptoms that patients could identify themselves are the presence of increased thirst (polydypsia), increased urination (polyuria) and increased hunger (polyphagia). Diabetes is diagnosed clinically by measuring the blood glucose level and glycated hemoglobin content. Patients with a fasting plasma glucose level of ≥ 126 mg/dl and glycated hemoglobin content of ≥ 6.5% are considered diabetic (NIDDK, 2005). The major factors that contribute to the pathogenesis of diabetes include genetic, environmental (diet, stress and lifestyle) and immunogenic as well as the use of several drugs (glucocorticoids, β-adrenergic agonists and statins) (Riserus and Willet, 2009).

1.2 TYPES OF DIABETES

The most common classes of diabetes are the type 1 and type 2 diabetes. Other forms of diabetes such as gestational, impaired glucose tolerance and diabetes related to malnutrition are relatively rare.

1.2.1 TYPE 1 DIABETES

It is also called as insulin dependent diabetes mellitus. It is an autoimmune disorder, in which there is loss of pancreatic beta cells, resulting in insulin deficiency (Rother, 2007). Approximately 10% of the diabetic cases are affected by type 1 diabetes. It is also termed as juvenile diabetes, because the onset of type 1 diabetes occurs at an early age, specifically affecting children. Patients with type 1 diabetes are supplemented daily with insulin in order to control the blood glucose level.
1.2.2 TYPE 2 DIABETES

It is also called as non-insulin dependent diabetes mellitus which accounts for more than 90% of the diabetic cases. It is often associated with obesity in which the cells do not respond to the insulin action. Type 2 diabetes was believed to have its onset only after 40 years, but recent studies have diagnosed it in young people too. Insulin administration is not mandatory in type 2 diabetic patients for survival, but they may become insulin dependent at advanced stages of the disease (Bennett, 2000).

1.3 DIABETIC COMPLICATIONS

Diabetic patients are prone to several acute complications such as diabetic ketoacidosis, hyperosmolar state, diabetic coma and periodontal diseases. Patients with uncontrolled blood glucose level i.e. prolonged hyperglycemia encounter deleterious effects in various organs such as eye, foot, skin, nervous system, kidney and heart (Esper et al., 2009). Because of the hyperglycemic condition, the endothelial cells lining the blood vessels take up more glucose through the insulin independent pathway. This results in the formation of more glycoproteins which lead to basement membrane thickening, thus affecting the blood vessels. When there is damage to the small blood vessels, they are grouped as microvascular complications and when the arteries are damaged, they are grouped as macrovascular complications. The macrovascular complications include coronary artery disease leading to heart attack, stroke and peripheral vascular disease (Koning et al., 2013). Atherosclerosis is considered as a major contributor for various cardiovascular diseases in diabetic patients. The major microvascular complications are;

*Retinopathy*: It is the damage to the retina of the eyes and is considered as the most common complications of diabetes. The chance of becoming blind is almost 25 times higher in diabetic patients compared to normal. The symptoms associated with retinopathy are loss of pericytes, venous dilations, endothelial cell proliferation with hyalinization and increased refractive power of lens. In most cases, damage to the eyes occurs in 5-10 years from the onset of diabetes (Romero-Aroca, 2011).

*Neuropathy*: It is the damage to the nerves that leads to foot ulceration and amputation. The frequency of neuropathy ranges between 5-60% depending on the patient’s age and
duration of diabetes. The symptoms associated with neuropathy are non-painful numbness of the toe and foot, burning pain, fullness of the skin of the toes, feet and legs, dysesthesia, fasciculation, difficulty in swallowing and speech impairment (Koning et al., 2013).

Nephropathy: It is the damage to the blood vessels and nephrons in the kidney that leads to renal failure. The kidney damage is predicted to occur between 15-25 years from the onset of diabetes. In type-1 diabetes, the progression to diabetic nephropathy is well defined, but in type-2 diabetes, it is less known because of the cardiovascular diseases associated with it.

1.4 DIABETIC NEPHROPATHY

Diabetic nephropathy (DNP) was first discovered by Clifford Wilson, a British physician (1906-1977) and Paul Kimmelstiel, an American physician (1900-1970) and the description of the disease was first published in 1936. Diabetic Nephropathy, also known as Kimmelstiel-Wilson syndrome is a chronic kidney disease caused by diabetes that leads to End Stage Renal Disease (ESRD). Though the pathophysiology of type-1 and type-2 diabetes is different, both the types of diabetes have an equal chance for progressing to nephropathy (Breyer et al., 2013). It is predicted that 25-40% of the diabetic patients eventually progress to nephropathy within a time span of 15-25 years from the diagnosis of diabetes. The mortality in diabetic patients with nephropathy is thirty times higher than patients without nephropathy (American Diabetes Association, 2004). Diabetic patients with poor blood pressure control and high cholesterol level are at high risk for progressing to nephropathy (Gross et al., 2005).

1.4.1 PREVALENCE OF DIABETIC NEPHROPATHY

Diabetic nephropathy is more prevalent among African Americans, Asians and Native Americans than Caucasians (Young et al., 2003). Asian subjects have significantly higher prevalence (52.6%) of diabetic end stage renal disease when compared to the Caucasians (36.2%) and migrant Asian Indians have 40 times greater risk of developing ESRD when compared to the Caucasians (Chandie-Shaw et al., 2002). In India, diabetic nephropathy accounts for no less than 46% of the chronic kidney diseases (Prakash et al.,
2006). Studies conducted by the Madras Diabetes Research Foundation (MDRF) in 2007 revealed that in urban Asian Indians, the prevalence of overt nephropathy and microalbuminuria was 2.2 and 26.9% respectively (Unnikrishnan et al., 2007). It is predicted that out of 30 million people living with diabetes in India, 6.6 million are expected to develop diabetic nephropathy by 2030 (Chandie-Shaw et al., 2006).

1.4.2 SYMPTOMS OF DIABETIC NEPHROPATHY

In the early stages of DNP, the disease shows no symptoms. Only at the later stages symptoms appear, as a result of excretion of high amount of protein in the urine. The initial histological changes in kidney include mesangial expansion, thickening of the glomerular basement membrane and podocyte loss. These changes further progress to glomerulosclerosis and tubulointerstitial fibrosis in the final stages of DNP (Brosius et al., 2009). Though several animal models have been established for DNP, they failed to develop glomerulosclerosis and tubulointerstitial fibrosis which are observed in the final stages of human DNP (Berthier et al., 2009). So there is a need for standardization of rat model for diabetic nephropathy.

1.4.3 PATHOLOGICAL STAGES OF DIABETIC NEPHROPATHY

The diabetic patients eventually progress to DNP through five different stages as illustrated in Mogensen classification (Mogensen et al., 1983). The first stage is the hypertrophy stage characterized by increased glomerular hyperfiltration and renal hypertrophy which occur at the diagnosis of diabetes or shortly thereafter. The second stage is the silent stage characterized by expanded mesangium with normal albumin excretion rate (AER). Since AER is normal with no prominent morphological changes, this stage of progression is considered clinically “silent”. With insulin treatment the changes in these two stages are, at least, partly reversible. The third stage is the incipient stage characterized by microalbuminuria with an albumin level of 30-300 mg/dl in urine (Table 1.1). It is considered clinically significant because the patients could be diagnosed only at this stage. Patients with incipient nephropathy are at high risk for progressing to the next stage, the overt nephropathy. The fourth stage is characterized with an albumin level of ≥ 300 mg/dl in urine, increased blood pressure and a decreased glomerular
filtration rate (GFR). This occurs within a time span of 15-25 years from the onset of diabetes. The final stage is the uremic stage characterized by end stage renal diseases (Robinson and Freedman, 2013).

<table>
<thead>
<tr>
<th>Stages</th>
<th>Characteristics</th>
<th>Albumin</th>
<th>GFR</th>
<th>Chronology (in years)</th>
</tr>
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<tbody>
<tr>
<td>Hypertrophy</td>
<td>Glomerular hyperfiltration</td>
<td>No increase</td>
<td>Increased</td>
<td>-</td>
</tr>
<tr>
<td>Silent stage</td>
<td>Expanded Mesangium</td>
<td>10-30 mg/dl</td>
<td>Normal</td>
<td>0 - 5</td>
</tr>
<tr>
<td>Incipient stage</td>
<td>Microalbuminuria</td>
<td>30-300 mg/dl</td>
<td>GFR begins to fall</td>
<td>6 - 15</td>
</tr>
<tr>
<td>Overt nephropathy</td>
<td>Macroalbuminuria</td>
<td>&gt; 300 mg/dl</td>
<td>GFR less than normal</td>
<td>15 - 25</td>
</tr>
<tr>
<td>Uremic</td>
<td>ESRD</td>
<td>May decrease</td>
<td>Decreased</td>
<td>25 - 30</td>
</tr>
</tbody>
</table>

Table 1.1: Different stages of progression of diabetic nephropathy
(Mogensen et al., 1983)

1.5 PATHWAYS MEDIATING THE PATHOGENESIS OF DNP

Hyperglycemia is considered as the major player in causing both microvascular and macrovascular complications. Hyperglycemia has been predicted to activate four different pathways such as polyol pathway, hexosamine pathway, protein kinase pathway and advanced glycation end product (AGE) formation (Rolo and Palmeira, 2006), which ultimately lead to various diabetic complications (Figure 1.1).
1.5.1 ADVANCED GLYCATION END PRODUCT FORMATION PATHWAY

Glycation reaction often referred as the Maillard reaction is the non-enzymatic binding of proteins and lipids to sugars. It was initially identified as the browning reaction associated with food spoilage, altered taste and loss of nutrition (Nagaraj et al., 2008). Glycation reaction is the nucleophilic addition of a free amino group of a protein to a carbonyl group of a reducing sugar, resulting in the labile Schiff base. This occurs over a period of hours, and once formed, rearranges to form a stable Amadori product. The Amadori product formation occurs over a period of days, and once formed are almost practically irreversible. The Amadori products are further decomposed, involving dicarbonly intermediates to form advanced glycation end products (Vistoli et al., 2013). The formation of advanced glycation end products, initiated by hyperglycemia and oxidative stress has been depicted in figure 1.2.

Figure 1.1: Pathways involved in the pathogenesis of DNP (Brownlee, 2001)
Mechanism of advanced glycation end products induced damage

In general, the AGE precursors damage the target cells through three different mechanisms (Brownlee, 2001).

1) Intracellular proteins modified by AGEs have altered function
2) Extracellular matrix components modified by AGE interact abnormally with other matrix components and with the receptors of matrix proteins. This affects both matrix to matrix and cell to matrix interaction
3) AGE modified proteins bind to AGE receptors on endothelial cells, mesangial cells and macrophages. This generates reactive oxygen species and activates the transcription factor NF-κB, leading to the pathological changes

In hyperglycemic condition, AGE formation occurs within a week in the endothelial cells. In endothelial cells, fibroblast growth factor and the proteins involved in macromolecular endocytosis are also modified by AGEs (Figure 1.3). Apart from this, significant matrix proteins such as collagen type-1, collagen type-4, laminin and fibronectin are also modified by AGEs (Forbes et al., 2003). This affects the network like structure that is formed because of the matrix-matrix interaction. AGEs are proven to be
chemotactic for monocytes both *in vitro* and *in vivo*. AGEs induce the migration of monocytes across the endothelial cell layer, through the activation of NF-κB. Apart from this, the binding of AGE to its receptor alters the cell structure and increases the permeability of macromolecules into the endothelial cells (Brownlee, 2001).

The AGEs are filtered by the glomerulus and are catabolized by the endolysosomal system in the proximal tubule of the kidney. AGE-receptor mediated mechanism triggers several cellular responses such as cytokine secretion and oxidation reaction (Gugliucci and Bendayan, 1995). In diabetes, activation of these cellular responses could mediate interstitial fibrosis along with glomerulosclerosis, which are the prominent histological features of end-stage renal disease (Sugimoto et al., 2007). In mesangial cells, AGEs stimulate platelet derived growth factor synthesis, which in turn stimulates the synthesis of matrix proteins such as collagen IV, laminin and fibronectin leading to mesangial matrix expansion and glomerulosclerosis (Forbes et al., 2003).

![Figure 1.3: AGEs bridging between endothelial cells and mesangial cells](Brownlee, 2001).
1.5.2 HEXOSAMINE PATHWAY

In the hexosamine pathway, fructose 6-phosphate is diverted from the glycolysis pathway and provides substrate for proteoglycan synthesis and for the formation of O-linked glycoproteins (Schleicher and Weigert, 2000). The rate limiting enzyme glutamine:fructose-6-phosphate aminotransferase is involved in the conversion of glucose to glucosamine. This further induces an increase in the transcription of TGF-α, TGF-β and PAI-I. Though the exact mechanism for the hyperglycemia induced gene transcription is not known, glucosamine itself has been shown to activate the PAI-I promoter in glomerular mesangial cells, paving way for the pathogenesis of DNP (Du et al., 2000).

1.5.3 INCREASED POLYOL PATHWAY

In the hyperglycemic condition, excess glucose is converted to sorbitol by the action of aldose reductase enzyme. Sorbitol thus formed is further oxidized to fructose by the enzyme sorbitol dehydrogenase. Flux through this pathway has been proven to contribute to several diabetic complications, though it has been found to be tissue specific. The detrimental effect of polyol flux is through increased osmotic stress, increase in cytosolic NADH/NAD$^+$ levels, decrease in cytosolic NADPH levels and decreased ATPase activity (Uehara et al., 2004). Sorbitol has been found to cause osmotic damage in microvascular cells, because it could not diffuse easily across the cell membrane. Increased polyol flux is found to decrease phosphotidyl inositol synthesis, thus causing a decrease in Na$^+$/K$^+$ ATPase activity. Increased polyol flux has also been found to have a direct correlation with protein kinase C (PKC) activation through the activation of triose phosphate and diacylglycerol (Uehara et al., 2004).

1.5.4 ACTIVATION OF PROTEIN KINASE C

In the hyperglycemic condition, PKC activation occurs primarily through the $de novo$ synthesis of diacylglycerol (DAG). The glycolytic intermediate dihydroxyacetone phosphate is reduced to glycerol-3-phosphate and through a stepwise acylation DAG is activated (Koya and King, 1998). PKC activation also occurs indirectly through the polyol pathway and AGE formation. During the formation of AGE, there is generation of
ROS. The ROS production activates phospholipase-D that hydrolyses phosphatidylcholines forming DAG (Portilla et al., 2000). In the polyol pathway, there is increased NADH/NAD\(^+\) ratio. NADH is found to inhibit the oxidation of triose phosphates (dihydroxyacetone phosphate, glycerol-3-phosphahate), thus contributing for the de novo synthesis of DAG (Graves and Kayal, 2008).

The multiple effects mediated by the activation of PKC have been depicted in figure 1.4. The activation of PKC mediates renal blood flow abnormalities such as decreasing nitric oxide production or increasing endothelin activity. The decreased nitric oxide production has been observed in the glomeruli and in the smooth muscle cells (Ganz and Seftel, 2000). In accordance with these reports, it has been proven in vitro in cultured endothelial cells that there is decreased endothelial nitric oxide synthase (eNOS) expression with the activation of PKC (Kuboki et al., 2000). The PKC activation increases the endothelin-1 stimulated Mitogen-activated protein kinase (MAPK) activation in glomerular mesangial cells and there is increased expression of VEGF in smooth muscle cells (Glogowski et al., 1999). This is reflected by the increased permeability of endothelial cells during hyperglycemic condition. Further, PKC activation induces the expression of matrix proteins and contributes for the mesangial expansion in DNP (Toyoda et al., 2004).
1.6 FACTORS INVOLVED IN THE PATHOGENESIS OF DNP

1.6.1 HYPERGLYCEMIA

Hyperglycemia is considered as the major player in causing diabetic complications. It mediates the progression of DNP through the formation of AGE, activation of PKC, increased polyol flux and increased hexosamine flux. Hyperglycemia is reflected by the formation of AGE and the level of AGE has a direct correlation with the risk of developing diabetic complications. Increased level of AGE in serum is an indication for the change in kidney morphology such as mesangial matrix expansion and glomerular basement membrane thickening (Vistoli et al., 2013).

1.6.2 HYPERTENSION

Hypertension is found to have a strong association with renal injury in diabetic patients. The onset of DNP is early in hypertensive patients compared to normotensive patients and the rate of progression to overt nephropathy is also higher in patients with hypertension (Ruggeneti et al., 2012). Several cross sectional and longitudinal studies
have found that high GFR is a risk factor for the development of DNP (Mogensen and Christensen, 1984; Rudberg et al., 1992, Bangstad et al., 2002). The blood pressure is regulated by the renin angiotensin aldosterone system (RAAS). The precursor angiotensinogen is converted into angiotensin I by the action of the enzyme renin, which is secreted from the juxtaglomerular cells. Angiotensin converting enzyme converts the inactive angiotensin I to the active form angiotensin II, which is an octapeptide. Apart from this, angiotensin converting enzymes (ACE) also degrades the vasodilator bradykinin into inactive form, thus increasing the blood pressure. Angiotensin II binds with two types of receptors, angiotensin II receptor type-1 and type-2, which are present in the adrenal cortex and kidney (Burns, 2000). The binding of angiotensin II (Ang-II) to its receptor increases the intraglomerular pressure by causing vasoconstriction in the renal vessels. Ang-II also stimulates the production of aldosterone hormone, contributing for the increased blood pressure. Further, Ang-II induces the synthesis of TGF-β and thereby stimulates mesangial matrix expansion (Ma et al., 2012), exacerbating the progression of DNP. ACE inhibitors and angiotensin II receptor blockers (ARB) have been found to be effective in treating DNP (Fujisawa et al., 2005; Ogawa et al., 2013), corroborating the importance of hypertension in DNP.

1.6.3 DYSLIPIDEMIA

Lipids in general are used for energy storage by the living system. They also act as signaling molecules and structural components of the cell. Lipoproteins, which are composed of lipids and proteins, are the transporters of lipids throughout the body. Of all the lipoproteins, low density lipoprotein (LDL) is called as ‘Bad Cholesterol’ because of its deleterious effects; it becomes more treacherous when oxidized (Dessi et al., 2013). The oxidized LDL (Ox-LDL) is implicated in the pathogenesis of various diseases such as atherosclerosis, cardiovascular diseases such as stroke, thrombosis, and endothelial dysfunction (Nilsson et al., 2007). Apart from these diseases, it has also been found to be involved in the pathogenesis of DNP. The oxidation of LDL does not occur when they are in circulation because of the presence of anti-oxidant enzymes (Holvoet et al., 2008). But, LDL becomes oxidized when a small portion of it that circulates in the plasma traverses the sub-endothelial space and gets engraved there (Dessi et al., 2013). The
reason why and how this LDL reaches the sub-endothelial space is yet to be explored. The free radicals that are released from the monocytes/macrophages, endothelial cells and smooth muscle cells oxidize LDL to form Ox-LDL. Increase in the level of serum Ox-LDL was observed in patients with chronic kidney diseases, including diabetic nephropathy (Kasahara et al., 2004).

Oxidized LDL is involved indirectly in the pathogenesis of DNP by inducing the expression of transforming growth factor- beta (TGF-β) (Nakhjavani et al., 2010). Activated TGF-β is proven to play a direct role in the thickening of the glomerular basement membrane (Rutledge et al., 2010). It binds to the TGF-β type-II receptor that phosphorylates type-I receptor which results in the activation of Smad proteins (Abe et al., 2011). This leads to the upregulation of extracellular matrix proteins such as collagen, laminin and fibronectin (Chen et al., 2001). Further, there is an increased level of protease inhibitors such as plasminogen activator inhibitor and inhibitor of metalloproteinases in diabetic condition (Eddy and Fogo, 2006). Thus, the net result of TGF-β activation is an increase in the synthesis of extracellular matrix proteins and a decrease in the degradation of extracellular matrix. This leads to extracellular matrix accumulation which leads to glomerulosclerosis thereby contributing to DNP (Figure 1.5).

TGF-β stimulates the expression of cyclin dependent kinase (CDK) inhibitors such as p27 and p21, thereby inhibiting the activity of cyclin dependent kinases (Chen et al., 2001). Thus the cells are arrested in the G1 phase that leads to cellular hypertrophy (Van-den-berg et al., 2003). Further, TGF-β enhances NOX-2 and NOX-4 (NADPH Oxidase isoforms) activities in kidney fibroblasts, which stimulate extracellular signal regulated kinase pathway (ERK-1) (Constant-Inescu et al., 2003). This results in the conversion of fibroblast into myofibroblast which is associated with kidney fibrosis (Bondi et al., 2010). In podocytes, the Ox-LDL is up-taken by the chemokine CXCL16, which is a membrane bound chemokine with solubilization property. Podocyte loss with damage in podocyte slit has been found to be associated with the oxidation of LDL (Nosadini and Tonolo, 2011).
Increase in the level of Ox-LDL is found to increase the level of VEGF expression and in turn stimulate endothelial cells to synthesize platelet derived growth factor (PDGF), which results in the activation of mesangial cells. The activated mesangial cells secrete CCL2, ICAM and VCAM (Cell Adhesion Molecules) which are involved in the recruitment of monocytes into the glomerulus (Chang et al., 2010). These recruited monocytes phagocytose oxidized lipoproteins that lead to the formation of foam cells in the glomerulus. Thus, the pathological changes such as glomerulosclerosis, mesangial expansion, macrophage infiltration, cellular hypertrophy and podocyte loss are mediated by oxidized LDL (Shiju and Pragasam, 2012).

Figure 1.5: Lipoprotein modification in the progression of DNP
(Shiju and Pragasam, 2012)
1.6.4 OXIDATIVE STRESS

Oxidative stress is caused by an imbalance in the production of oxidants versus antioxidant enzymes. The imbalance in the oxidant/antioxidant equilibrium results in the increased level of ROS. ROS include singlet oxygen, hydrogen peroxide, and free radicals such as superoxide and hydroxyl ion. ROS are generated by the electron transport chain, cytochrome P450, xanthine oxidase, polyol pathway and NADPH Oxidase (Dusting and Triggle, 2005). In normal physiological condition, ROS are involved in cell signaling and homeostasis. ROS are generated to combat infection and are produced in response to proliferation stimulated by growth factors.

The interplay between hyperglycemia, oxidative stress and diabetic complications has been proven by several experimental and clinical studies (Fiorentino et al., 2013). There is also evidence that increased oxidative stress contributes to the pathogenesis of DNP and its progression to ESRD (Tripathi and Yadav, 2013). ROS generation has been linked to vasoconstriction, endothelial dysfunction, increased sodium reabsorption and modification of matrix proteins. Oxidative stress activates the transcription factors NF-κB and activator protein-1, that leads to the transcription of genes encoding cytokines, growth factors and matrix proteins. An increase in inflammatory cytokines and growth factors further exacerbates the oxidative stress (Dusting and Triggle, 2005). Though oxidative stress derived inflammation has been found to mediate DNP, elucidating the association between oxidative stress and DNP has been a difficult task.

1.6.5 DIETARY PROTEIN INTAKE

Earlier in 1952, it was found that the consumption of protein based diet increased the renal blood flow and GFR. It was found that the protein rich diet increased the renal workload and contributed for the development of kidney damage. Lowering the protein intake has been practiced as a treatment for chronic kidney diseases. While the recommended protein intake for diabetic patients without microalbuminuria is 56-111 g/kg body weight, the recommended protein intake for diabetic patients with microalbuminuria is only 0.6-0.8 g/kg body weight (Velazquez-lopez et al., 2008). This signifies the importance of protein intake as a risk factor for the progression of DNP.
1.6.6 SMOKING

Smoking has been considered as a risk factor for DNP and several studies have proven the association between the prevalence of DNP and smoking (Chakkarwar, 2012). Smoking causes an increase in the blood pressure with an increase in the level of aldosterone and cortisol. This causes a change in the renal hemodynamic system, contributing to the kidney damage (Agarwal, 2005). Oxidative stress is considered as a possible link between smoking and DNP. Hyperglycemia induced ROS generation is more pronounced by the oxidative stress caused by smoking, thus exacerbating the pathological changes in DNP (Makuc and Petrovic, 2011).

1.7 DIAGNOSIS OF DNP

Diabetic nephropathy is characterized by the presence of abnormal amount of protein in the urine, because of the alteration in the renal filtration capabilities. So the presence of albumin in urine has been considered as a biomarker for the progression and development of DNP (The microalbuminuria collaborative study group, 1999; Royal college of Physicians in Edinburgh Diabetes register group, 2000). However, microalbuminuria is considered as a non specific biomarker because only 30-45% of microalbuminuric type-2 diabetic patients eventually develop overt proteinuria (Caramori et al., 2000) and microalbuminuria has also been proposed as a biomarker for cardiovascular risk in diabetic and non diabetic patients (Mancia et al., 2009). Perkins et al., (2007) have found that the problem with using albumin as a biomarker is twofold: Low grade albuminuria is a poor predictor of the diseased state and high grade albuminuria develops at a stage when nothing can be done to prevent the progression of DNP (Perkins et al., 2007).

Various high-throughput screening methods are being used to find biomarkers for DNP with the advancement in various fields like genomics, proteomics and metabolomics. Genetic trials including the Family Investigation of Nephropathy and Diabetes [FIND] and Genetics of Kidneys in Diabetes [CoKinD] have been conducted to find the genetic polymorphism associated with DNP. These trials identified multiple loci, associated with increased risk of diabetic nephropathy, indicating that it is a polygenic
disease with multiple genes involved with small effects (Mueller et al., 2006; Iyengar et al., 2007). Though this method seems robust enough to find biomarkers, it requires kidney biopsy sample for its analysis. In current clinical practice, it is highly cumbersome to obtain kidney biopsies from diabetic patients (Kato et al., 2008).

Proteomics and lately metabolomics hold the greatest promise for identifying a novel biomarker for DNP (Thongboonkerd, 2011; Wettersten and Weiss, 2013). Various body fluids like blood, urine, cerebrospinal fluid, and tissues are being used for the proteome analysis. However, the proteome is highly complex and variable in all these body fluids (Thongboonkerd, 2011). Many biologically interesting molecules are low abundant proteins whereas the 22 most abundant proteins such as albumin, tamm horsfall protein, transferrin, haptoglobin, globulins and etc. constitute 99% of the total protein mass in blood and urine (Kato et al., 2008). These highly abundant proteins mask the less abundant proteins and restrict us from identifying the biomarker (Mischak et al., 2009). Ben-Ameur et al. (2010) have reviewed the 22 works that have been done on the proteome analysis in biomarker discovery for DNP and reported that 34 different proteins are up-regulated and 34 down-regulated. Also it has been observed that there is a very low consistency in the proteins identified. This proves that no clear picture can be obtained on the biomarker discovery by the proteome analysis. Metabolomics also gained increased attention because the human metabolome consists of 3000 molecules which are by many orders of magnitude ($10^5$) lower than the number of transcripts and the proteins (Kouskoumvekaki and Panagiotou, 2011). However it doesn’t prove to be effective for biomarker discovery in DNP.

Several proteins such as N-Acetyl glucuronidase (NAG), Neutrophil gelatinase-associated lipocalin (NGAL), Kidney injury molecule-1 (KIM-1), Smad 1, Cystatin C, and adhesion molecules such as ICAM, VCAM have been identified as biomarkers for DNP (Ben-Ameur et al., 2010). Because of several disadvantages, these proteins are not used in clinical practice. Since these proteins are not involved in the pathophysiology of DNP, the most common problem associated with these proteins as biomarker for DNP is their non-specificity. Our study has explored the expression level of Cluster of
differentiation 36 (CD36) in diabetic nephropathy, to identify if it could serve as a possible marker for DNP.

1.8 CLUSTER OF DIFFERENTIATION 36 (CD36)

CD36 (Cluster of Differentiation 36) is a member of class B scavenger receptor family of cell surface proteins and is known by diverse names such as FAT (Fatty Acid Translocase), SCARB3 (Scavenger receptor class B-member 3), GP88 (Glycoprotein 88), GPIV (Glycoprotein IV), GPIIIb (Glycoprotein IIIb), Leukocyte differentiation antigen CD36, Platelet glycoprotein IV and Cluster determinant 36. Being a scavenger receptor, CD6 could bind and interact with several ligands including advanced glycation end products, thrombocytes, erythrocytes parasitized with *Plasmodium falciparum*, native lipoproteins, collagen (type-1 and IV), Ox-LDL, oxidized phospholipids, platelet agglutinating protein and long chain fatty acids (Silverstein and Febbraio, 2009). CD36 is an 88 kDa membrane glycoprotein that was first identified on monocytes using monoclonal antibody OKM5 (Talle et al., 1983) and then subsequently isolated from platelets (Asch et al., 1987). CD36 is expressed in various cell types such as platelets, erythrocytes, monocytes, differentiated adipocytes, mammary epithelial cells, spleen cells and microdermal endothelial cells.

1.8.1 GENE ENCODING CD36

The human CD36 gene is more than 46 kb in size and is located on the band q11.2 of chromosome 7 (Armesilla et al., 1987). Out of the 15 exons, only part of exon 3, exons 4-13 and part of exon 14 code for the CD36 protein (Collot-Teixeira et al., 2007). Though exon 1 and 2 are not translated, they are found to contain the proximal promoter region. Two alternative exons 2a and 2b have also been identified in the untranslated region. The start codon has been identified 290 bp downstream to the transcriptional start site in exon 3. The N-terminal intracellular and the transmembrane regions are encoded by exon 3 and the C-terminal intracellular and the transmembrane regions are encoded by exon 14. In exon 14, there is an internal splice donor site that could join with exon 15, generating a shorter CD36 transcript (Cheung et al., 2007). Though the significance of the alternative transcripts is not known, they don’t affect the coding region. The gene structure of CD36
is well characterized in human, rat, mice and is found to have high level of identity among the three orthologues (Rac et al., 2007).

1.8.2 CD36 PROTEIN STRUCTURE

CD36 is a 472 amino acids polypeptide (UniProtKB/Swiss-Prot P16671) which is predicted to adopt a ditopic configuration with an extracellular domain, flanked by two transmembrane and two cytoplasmic domains. The extracellular region is rich in N-glycosylation sites (Tandom et al., 1989) that bears a hydrophobic domain (amino acids between 184-204) which helps in interacting with the plasma membrane (Figure 1.6). The region spanning from 155-183 is considered as the binding site that could bind with the ligands Ox-LDL (Puente et al., 1996) and advanced glycated end products (Ohgami et al., 2001). Though the mathematically predicted molecular weight of CD36 polypeptide was 53 kDa, the actual molecular weight of CD36 was found to be approximately 88 kDa when observed in SDS-PAGE (Greenwalt et al., 1990). The difference in molecular weight is attributed to the post translational modifications of the protein. There are 10 N-glycosylation sites, 8 of which are conserved between rat and human. However, all the 10 cysteine residues are found to be conserved between both rat and human (Abumrad et al., 1993). Six cysteine residues are clustered in the C-terminal region of the extracellular loop forming three disulphide bonds. Inhibition of disulphide bond formation may prevent the maturation of CD36, degrading it in the endoplasmic reticulum. The remaining four cysteine residues which are present at the ends are modified by palmitoylation (Tao et al., 1996). The tertiary structure of CD36 was predicted using Kyte-Doolittle analysis and was found to have hydrophobic regions near each end large enough to span the cellular membrane.

1.8.3 FUNCTIONS OF CD36

In general, CD36 is identified as a scavenger receptor and is involved in uptake of AGE, Ox-LDL, oxidized phospholipids, long chain fatty acids and collagen. It is involved in the recognition and cytoadherence of Plasmodium falciparum infected erythrocytes (Silverstein and Febbraio, 2009). In association with thrombospondin (TSP1), it is involved in inhibition of angiogenesis, which is the body’s response to
vascular injury and ischemia (Dawson et al., 1997). The CD36-TSP1 binding is also involved in anti-inflammatory pathway by up-taking apoptotic cells. The uptake of apoptotic cells is considered as the ancient function of CD36, which was revealed by the functional analysis of CD36 homologues in fly, worm and sponge (Febbraio and Silverstein, 2007). CD36 has been found to be involved in the phagocytosis of gram positive bacteria (Hoebe et al., 2005). There has been evidence that CD36 binds with the non-protein repetitive molecule present in cell membrane of bacteria. Though the exact ligand that could bind with CD36 is not known, there have been reports that it could bind either with lipoteichoic acid or lipoprotein present in bacteria (Hashimoto et al., 2006). This has further been substantiated by the finding that CD36 knockout mice are less efficient in the phagocytosis of bacteria, thereby less proficient in clearing pathogens (Stuart et al., 2005).
Figure 1.6: Gene and protein structure of CD36

(Collot-Teixeira et al., 2007, Silverstein and Febbraio, 2009)
1.9 INVOLVEMENT OF CD36 IN DIFFERENT DISEASED STATES

1.9.1 ROLE OF CD36 IN DYSLIPIDEMIA

Modification of LDL decreases the binding of LDL to its receptor and there is increased propensity for it to bind to scavenger receptors. LDL can be modified \textit{in vitro} using several agents. LDL oxidized using copper has been found to have high affinity for CD36 (Puente Navazo et al., 1996). Though the copper oxidation yields a high affinity binding lipoprotein, the level of oxidation might be higher compared to the lipoprotein that is modified \textit{in vivo} (Jenkins et al., 2004). Though there are contradictory reports, CD36 recognizes and binds Ox-LDL through oxidized phospholipid component or by binding to the phospholipid attached to apoB (Puente et al., 1996). The binding of Ox-LDL to CD36 mediates several pathways through the activation of NF-κB. The major pathological consequence of Ox-LDL binding to CD36 is the formation of foam cells that ultimately results in atherosclerosis (Febbraio and Silverstein, 2007).

1.9.2 CD36 MEDIATES ATHEROSCLEROSIS

When macrophages are exposed to Ox-LDL, there is activation of peroxisome proliferator activated receptor-gamma (PPAR-γ) which results in the increased expression of CD36 (Nagy et al., 1998). Because of the increased expression of CD36, there is cholesterol accumulation in the macrophages and are converted into foam cells (Febbraio and Silverstein, 2007). Apart from cholesterol accumulation, there is increased production of pro-inflammatory cytokines and other inflammatory mediators (Han et al., 1997). In human, high level of CD36 has been reported in foam cells of atherosclerotic plaques. CD36 expressed in endothelial cells and platelets are also found to contribute for plaque formation. CD36 mediates the adhesion of phagocytes to endothelial cells and it acts as a bridge between the monocytes and endothelial cells (Collot-Teixeira et al., 2007). In endothelial cells, binding of Ox-LDL to CD36 promotes the cholesterol efflux and there is internalization of eNOS. This induces a prothrombotic environment that could aggravate vascular dysfunction (Uottenbogaard et al., 2000).
1.9.3 CD36 AND INSULIN RESISTANCE

The functional role of CD36 as fatty acid translocase makes it important in insulin resistance. It has been reported that there is decreased fatty acid uptake in CD36 deficient mice (Goldberg et al., 2009). Because of the decreased fatty acid uptake, the cells depend on alternate substrate for energy, leading to hypoglycemia. When glucose was administered intraperitoneally in CD36 knockout mice, they did not show insulin resistance and there was a slightly higher whole body glucose uptake and lower glucose storage at basal condition. Though CD36 deficiency does not lead to diabetes in mice, there is evidence for hepatic insulin resistance (Goudriaan et al., 2003). In rats, when the CD36 gene was mutated, the rats resembled a spontaneously hypertensive rat model. These rats were found to be hypertensive, insulin resistant with impaired fatty acid uptake (Pravenec et al., 1999). In human, polymorphisms in CD36 associated with insulin resistance have been identified (Corpeleijn et al., 2006), but the mechanism by which CD36 impacts insulin resistance remains unclear. In a study conducted by Handberg et al., (2006) there was increased plasma level of soluble CD36 in diabetic patients with insulin resistance. It was also reported that the level of CD36 correlated positively with IL-6, which implies that CD36 is released into circulation as a part of pro-inflammatory state observed in insulin resistance.

1.9.4 CD36 AND INFLAMMATION

The binding of Ox-LDL to CD36 induces the activation of NF-κB, thus inducing the production of pro-inflammatory cytokines. The involvement of CD36 in inflammation has been reported in several studies (Okamura et al., 2009; Cai et al., 2012). Treatment of peripheral blood mononuclear cells (PBMC) with macrophage colony stimulating factor has been found to induce a 7 fold increase in CD36 m-RNA expression (Yesner et al., 1996). Further, cells treated with TNF-α showed increased surface expression of CD36 in monocytes (Nakagawa et al., 1998). In accordance with this finding, monocytes treated with IL-10, which is an anti-inflammatory cytokine, inhibited the up-regulation of CD36 through reduced PPAR-γ expression and increased cholesterol efflux protein ATP-binding cassette transporter protein (ABCA1) (Rubic and
Lorenz, 2006). Exposure of PBMC to IL-4 induces a 4 fold increase in CD36 m-RNA and an 8-10 fold increase in CD36 protein expression (Yesner et al., 1996).

1.9.5 IMPACT OF CD36 IN CARDIOVASCULAR DISEASES

CD36 is considered to play an important role in choosing the fuel substrate in heart (Kuang et al., 2004). Heart, which generally derives energy from fatty acids, relies mostly on glucose in the absence of CD36. This was confirmed by the observation that CD36 knockout mice encountered fasting hypoglycemia (Coburn et al., 2000). Since they rely on glucose, CD36 knockout mouse heart is protected during ischemic condition. The amelioration of ischemic injury in CD36 knockout mice was attributed to the decreased generation of ROS (Cho et al., 2005). Reactive oxygen and nitrogen species generated by leukocytes contribute for the generation of ligands for CD36. CD36 is the major protein involved in fatty acid uptake in cardiac muscle cells which was confirmed using specific CD36 inhibitor, sulpho-N-succinimidyl oleate. The uptake of fatty acids by CD36 indirectly causes cardiovascular diseases (Koonen et al., 2005).

1.10 POSITIVE FEEDBACK MECHANISM OF CD36

CD36 exists as a monomer unless it is recognized by a ligand. Whenever a ligand binds to CD36, it is endocytosed through a raft-mediated pathway. Through the PKC pathway, PPAR-γ is trans-activated and forms a heterodimer with retinoid X receptor (RXR). This PPAR-RXR complex binds to the PPAR response element in the CD36 promoter region thus activating CD36 expression (Collot-Teixeira et al., 2007). This indicates that through a positive feedback mechanism, with the increase in ligand there is an increase in the expression of CD36 (Figure 1.7).
1.11 CD36 IN THE PROGRESSION OF DNP

CD36 has been predicted to play an important role in the progression of diabetes to DNP. Because of the hyperglycemic condition maintained in diabetes, there is formation of glycated proteins which could bind to the receptor protein CD36. This activates the serine-threonine kinases Lyn and Fyn, which in turn activate the protein kinase C and MAPK pathway (Figure 1.8). This ultimately results in the activation of caspases, which leads to the apoptosis of proximal tubular epithelial cells (Susztak et al., 2005). Proximal tubular epithelial degeneration is considered as a hallmark in the progression of DNP. Since CD36 has been proven to be involved in the pathological mechanism of DNP and is also up-regulated with the increase in ligand, our study was designed to analyze the expression level of CD36 in different stages of DNP. In addition, the effect of treatment including insulin, aminoguanidine and aged garlic extract in preventing the progression of DNP has also been analyzed.
CONVENTIONAL TREATMENTS FOR DNP

Hyperglycemia, hypertension and dyslipidemia are considered as the major risk factors for diabetic nephropathy (Vistoli et al., 2013). In diabetic patients, these risk factors accelerate the rate of progression of diabetes to diabetic complications. Since diabetic nephropathy is a multi-factorial disease, the therapeutic interventions are targeted towards different pathological mechanisms and are aimed at retarding the progression.

1.12.1 ANTI-HYPERGLYCEMIC AGENTS

The anti-hyperglycemic agents are targeted towards a controlled blood glucose level. Since hyperglycemia is the major risk factor that activates diverse pathological
mechanisms, controlling the blood glucose level has been proven to be effective in preventing the progression of DNP. Tight glucose control (HbA1C levels < 6.5%) versus regular control (8-9%) demonstrated 39 and 54% lower rates of development of microalbuminuria and macroalbuminuria respectively (DCCT, 1993). Insulin is the most commonly used anti-diabetic drug and are available in different forms.

- Rapid-acting insulin: insulin lispro (Humalog®), insulin aspart (Novolog®)
- Short-acting insulin: insulin regular
- Intermediate-acting insulin: insulin NPH
- Long-acting insulin: insulin glargine (Lantus®), insulin detemir (Levemir®)

1.12.2 ANTI-GLYCATION AGENTS

Prolonged hyperglycemia leads to the formation of advanced glycation end products. AGEs have the ability to crosslink with different proteins, affecting their function and inhibiting different physiological mechanisms (Negre-Salvayre et al., 2008). Agents that directly inhibit or degrade AGEs have been successful in retarding the progression of DNP in animal models and are being developed for human clinical trials (Vasan et al., 2001). Since the formation of AGE is a multi-step process, the AGE inhibitors are targeted towards different products and are classified into six types (type A-F inhibitors).

Type A inhibitors act as sugar competitors by reacting with free amino groups of protein and prevent them from binding to sugars. They are also called as amino group capping agents. Examples of type A inhibitors are aspirin and the aldehyde pyridoxal-5'-phosphate. Type B inhibitors block the formation of cross-links by reacting with aldose and ketose sugars, inactivating them before they react with amino groups of proteins. Type B inhibitors include aminoguanidine, metformin, thiamine, benfotiamine and cinnulin (Rahbar and Figarola, 2003). Aminoguanidine, which is a nucleophilic hydrazine derivative with structural similarity to L-arginine is the most commonly used anti-glycation agent. Aminoguanidine contains a terminal amino group, which is of higher chemical reactivity than terminal amino group of proteins. It was shown to prevent lipid peroxidation in human plasma and red blood cell membrane as well as rat tissues. It is also known to be a selective inhibitor of inducible nitric oxide synthase (iNOS).
Aminoguanidine is proven to retard the progression of diabetic complications in diabetic rats (Soulis et al., 1996).

Type C inhibitors include metal chelators and antioxidants. Examples for chelators include diethylenetriamine-pentaacetic acid, phytate, deferoxamine and penicillamine. Antioxidants include vitamin C, E and lipoic acid which possess good inhibitory activity. Type D inhibitors trap reactive dicarbonyl intermediates (glyoxal, methyl glyoxal, glycolaldehyde, and glucosones) to form substituted triazines. Type D inhibitors include aminoguanidine, metformin, carnosine and L-arginine (Rahbar and Figarola, 2003). Type E inhibitors, also called as Amadori adducts inhibitors, prevent formation of AGE from Amadori products. They include aminoguanidine, and also compounds that have potential for enzymatic deglycation at the Amadori level such as carnosine and metformin. Type F inhibitors, also called as cross-link breakers, have the ability to break the cross-links even after they are formed. Type F inhibitors include ALT-711, N-phenacylthiazolium bromide, vitamin B1 and its synthetic fat-soluble form benfotiamine (Vasan et al., 2001).

1.12.3 ANTI-HYPERTENSIVE DRUGS

Since hypertension accelerates the progression of DNP, drugs that could reduce the blood pressure are effective in retarding the progression of DNP. It was evinced that patients with a blood pressure of 144/97 mm Hg had an estimated loss of GFR of 0.91 ml/min/month. When the same patients were treated with an antihypertensive drug regimen to attain an average blood pressure of 128/84 mm Hg using a diuretic, β-blocker and vasodilator, the rate of loss of GFR was reduced by ~ 57% to 0.39 ml/min/month (Appel, 2013). Statins such as atorvastatin, lovastatin, pitavastatin and pravastatin have been found to be beneficial in ameliorating DNP (Strippoli et al., 2008).

1.12.4 INHIBITION OF THE RAAS SYSTEM

ACE inhibitors prevent the conversion of inactive AngI to its active form AngII. Thus they are proven to decrease GFR, ameliorating microalbuminuria in DNP. ACE inhibitors that are frequently used include ramipril, captopril, enalapril and lisinopril (Burns, 2000). Along with ACE inhibitors, angiotensin receptor blockers such as
olmesartan, candesartan, irbesartan and losartan are prescribed for inhibiting the RAAS system. ARB prevents the binding of AngII to its receptors, thus decreasing the intraglomerular pressure. This further inhibits the secretion of aldosterone hormone, thus attenuating the blood pressure. ARB blockers are proven to improve mesangial expansion in DNP (Ogawa et al., 2013). Apart from these commercial drugs, interest has been focused on plant based drugs with more efficacies and less side effects. Several plants such as Trigonella foenum (Fenugreek), Brassica oleracea (Red cabbage), Prunella vulgaris (Heal-all), Ephedra sinica (Brigham Tea), Curcuma longa (Turmeric), Psidium guajava (Common Guava), Caesalpinia sappan (Sappanwood) and Trifolium alexandrium (Egyptian Clover) have been explored for their potential in preventing the progression of DNP (Musabayane, 2012).

1.13 AGED GARLIC EXTRACT

Garlic is a culinary herb with lot of medicinal properties. The potential health benefits of garlic and its components are well characterized. Among all components of garlic, organosulphur compounds are found to be very effective. The major sulphur compounds involved in the protection of cardiovascular disorders and diabetes include S-allyl cysteine sulphone (alliin), diallyl thiosulphinate (allicin), diallyl disulfide and γ-glutamyl-S-alkyl-L-cysteine (Rahman and Lowe, 2006). These sulphur compounds present in garlic are segregated into two groups based on their solubility as water soluble and oil soluble sulphur compounds. Intact garlic bulbs and certain garlic powders contain high amount of γ-glutamyl-S-alkyl-L-cysteine which are hydrolysed and oxidized to form alliin. Alliin accumulates naturally during prolonged storage of garlic bulbs. However, when garlic bulbs are crushed or damaged, the vacuolar enzyme alliinase transforms water soluble sulphur compounds such as alliin into oil soluble compounds such as allicin (Yan et al., 2013). Allicin is rapidly degraded into vinyldithiins and ajoenones depending on the conditions like concentration, temperature and pH. Though the oil soluble sulphur compounds are found to have anti-microbial property, they do not show potential hypolipidemic and anti-hyperglycemic properties (Amagase, 2006). So the water soluble sulphur compounds are preferably being used and are retained in the garlic by the process of ageing.
1.14 BENEFICIAL EFFECTS OF AGED GARLIC EXTRACT

Aged garlic extract has the ability to inhibit lipoprotein peroxidation and to scavenge hydroxyl and oxygen radicals. There is also evidence that aged garlic extract prevents the gentamicin-induced increase in renal 3-nitrotyrosine, an index of nitrosative stress, thereby protecting the tissue from nitrosative damage (Maldonado et al., 2003). Furthermore, aged garlic extract and S-allyl cysteine were able to prevent hemolysis (Morihara et al., 2001).

1.14.1 ANTI-OXIDANT PROPERTY

Reactive oxygen species generation is a significant pathological change that occurs in most of the diseased states and diabetes is no exception. In diabetic condition, free radicals are mostly generated because of the non-enzymatic and auto-oxidative glycosylation. Apart from this, change in energy metabolism and inflammation also contribute for the generation of free radicals (Anwar and Meki, 2003). So the anti-oxidant property of aged garlic extract is important in preventing the diabetic complications (Maldonado et al., 2003). In diabetic rats supplemented with garlic, there is increased plasma level of thiols, ceruloplasmin, albumin, and anti-oxidant enzymes such glutathione S-transferase (GST) and superoxide dismutase (SOD) (Krishnaswamy, 2008) with decreased lipid peroxidation (Anwar and Meki, 2003). This confirms the anti-oxidant property of aged garlic extract.

1.14.2 HYPOLIPIDEMIC PROPERTY

Aged garlic extract is found to decrease the rate of glycerol incorporation into triacylglycerol, diacylglycerol and phospholipid indicating that garlic has the potential to inhibit the synthesis of triacylglycerol and fats (Ali et al., 2000). Aged garlic extract has the ability to inhibit HMG-CoA reductase enzyme, which is a rate controlling enzyme in the synthesis of cholesterol (Liu and Yeh, 2002). Thus, there is decreased serum cholesterol level because of garlic supplementation. Supplementation of aged garlic extract in diabetic rats was found to decrease the level of triglycerides, cholesterol and it was found that the hypolipidemic effect of garlic is through the inhibition of lipoxygenase and cyclooxygenase enzymes (Ali et al., 2000).
1.14.3 HYPOTENSIVE PROPERTY

An imbalance in the formation of the vasoconstrictor thromboxane A2 (TXA2) and vasodilator prostacyclin (PGI2) facilitates thrombus formation, thus contributing for the increased blood pressure. Cholesterol seems to increase platelet aggregation by stimulating platelet TXA2 formation (Ali et al., 2000; Krishnaswamy, 2008). Apart from promoting platelet aggregation, TXA2 constricts blood vessel at the site of its release. Aged garlic extract enhances PG12 synthesis and lowers the cholesterol level. Thus this imbalance of TXA2 and PGI2 could be restored by the dietary intake of garlic (Tapiero et al., 2004). Further, ACE inhibition by garlic extract might also contribute for the antihypertensive activity.

1.14.4 HYPOGLYCEMIC PROPERTY

The hypoglycemic effect of aged garlic extract has been proven by several investigators (El-Demerdash et al., 2005, Saravanan et al., 2009). Garlic is proposed to have insulin-like substances present in it, which renders hypoglycemic effect. Other possible mechanisms include inhibition of insulinase activity, enhancing insulin secretion and inducing regeneration of beta cells in the pancreas. Further, the fibers present in garlic may interfere with carbohydrate absorption, thereby affecting blood glucose level. Garlic has also been proven to increase the glycogen content of the liver (El-Demerdash et al., 2005). In diabetic rats supplemented with aged garlic extract, there is enhanced glucose uptake in liver for the conversion of glycogen. Further, there is increased blood flow to the endocrine and exocrine pancreas, thus increasing the transport of insulin to target tissues (Liu et al., 2007). However, the hypoglycemic effect of garlic in human is not yet documented.

1.14.5 ANTI-GLYCATION PROPERTY

The anti-glycation property of aged garlic extract has been proven in vitro and the key ingredient responsible for the anti-glycation property was found to be S-allyl cysteine (Ahmad and Ahmed, 2006). Bovine serum albumin and lysozyme were glycated in vitro in the presence of glucose and methylglyoxal and at different concentrations of aged garlic extract and S-allyl cysteine. Both aged garlic extract and S-allyl cysteine inhibited
the formation of advanced glycation end products and showed potent amadorin activity (Ahmad et al., 2007). S-allyl cysteine was also found to inhibit the formation of N-(carboxymethyl) lysine (CML), an advanced glycation end product derived from oxidative processes (Ahmad and Ahmed, 2006). Similarly, four organosulphur compounds derived from garlic, diallyl sulfide, S-ethyl cysteine, S-allyl cysteine, and N-acetyl cysteine have been proven to protect LDL against oxidation and glycation. This confirms the potential of aged garlic extract in preventing the diabetic complications (Balamash et al., 2012).

Since the renoprotective effect of aged garlic extract has not yet been explored, this study was carried out to explore the efficacy of aged garlic extract in preventing the progression of nephropathy. Further, the effect of treatments such as insulin, aminoguanidine and aged garlic extract on CD36 expression has also been elucidated in this study.
1.15 AIM AND OBJECTIVES

Aim

The aim of the present study is to analyse the expression level of cluster of differentiation 36 (CD36) in experimental diabetic rats and humans progressing to nephropathy.

Objectives

- To standardize a rat model for diabetic nephropathy
- To analyze the m-RNA and protein expression level of CD36 in diabetic rats progressing to nephropathy
- To analyze the level of soluble CD36 in plasma and urine of diabetic patients with different stages of nephropathy
- To analyze the effect of treatments on the level of CD36 expression in preventing the progression of nephropathy