Diabetic nephropathy is amongst the leading cause of end-stage kidney disease (*Krolewski M et al., 1996* and *Bojestig M et al., 1994*), and has increased the morbidity and mortality in type 2 diabetic patients. The major devastating alterations of diabetic nephropathy (DN) remain unnoticed due to a prolonged period of clinical silence (*Mauer S et al., 1984*). Thus, the present scenario heralds some unsolved problems of diabetic nephropathy for defining a diagnosis in an early stage of nephropathy. Furthermore, microalbuminuria, an increase in creatinine and decrease in creatinine clearance reflects well established glomerular damage and hence, it is necessary to consider the pathological and clinical findings in more preliminary stages. Renal biopsy and creatinine clearance by exogenous markers are still considered more reliable; however, it is impossible to do such painful and time-consuming procedures in all cases. Thus, considering all aspects it becomes now necessary and significant for establishing non-invasive and reliable diagnostic biomarkers predicting diabetic nephropathy in its preliminary stage. The global scenario of diabetes is graphically represented in Fig 1.1.

**Figure 1.1 Global scenario of diabetes and its prevalence in future years**

(in millions)

India stands second in the global map of diabetes and it is predicted American diabetes association that in 2035 more than 130 million population will be affected by type 1 or type 2 diabetes (American Diabetes Association, Global atlas, 2011).

In India approximately 20-40% of the T2DM population will progress to overt nephropathy over 15 yrs of the period. But, now with increasing number of T2DM population, individuals with complications are also increasing (*Dronavalli S et al., 2008*). Guidelines of American
Diabetes Association refer to the diagnosis of DN soon after the identification of type 2 diabetes mellitus (T2DM) ([Diabetes Care, 2014](#)). A large portion of patients diagnosed with T2DM are accounted for having microalbuminuria and overt nephropathy ([Luiza M et al., 2000](#)) because diabetes is present for many years before the diagnosis is made ([Diabetes Care, 2004](#)). In 2013 India had approximately 65.1 million populations affected with diabetes and by 2035 it is estimated to affect approximately 109 million population ([Ekinci E et al., 2013](#)). The Indian scenario of diabetes affected population is depicted in Fig 1.2.

**Figure 1.2 Indian scenario of percent population affected by diabetes**

In Chandigarh 0.12 million, Jharkhand 0.96 million, Maharashtra (9.2 million) and Tamil Nadu 4.8 million are estimated to be affected by diabetes. The present scenario is not stable and is gradually increasing ([Kaveeshwar et al., 2014](#)).

The degree of renal function decline upon established nephropathy is highly variable amongst patients because it is highly influenced by additional factors like blood pressure and glycemic control. Multiple mechanisms contribute to the development and outcomes of diabetic nephropathy, such as an interaction between hyperglycemia-induced metabolic/hemodynamic changes and genetic predisposition, which sets a platform for kidney injury (Fig 1.3) ([Ziyadeh F et al., 2004](#)).
Exposures to risks like genetic and environmental (hyperglycemia) leads to various structural and functional alterations in kidney. The present situation detects the advanced manifestation (microalbuminuria) and then appropriate therapy is given. However, the necessity is prediction of earliest manifestation in reversible stages to prevent further alterations (Lin C et al., 2006).

Traditionally DN was considered as a primarily glomerular disease but now it is widely accepted that tubular damage initiates the functional alteration and decline in renal functioning significantly correlates with tubular damage (Mauer S et al., 1984 and Bohle A et al., 1991). DN is divided into distinct developmental phases (i) structural changes and (ii) functional alterations (Fig 1.4). Cell culture studies have provided insight into mechanisms of growth. Renal cells, when exposed to high glucose concentrations, shows that proximal tubular cells primarily undergo hypertrophy rather than in the glomerular compartment, where there is a self-limited proliferation of mesangial cells with subsequent hypertrophy (Wolf G et al., 1999). Tubuloepithelial renal cell hypertrophy and tubular basement membrane thickening occur very early in the course of diabetic nephropathy. Such changes are most likely the precursors of the later irreversible changes of tubulointerstitial cells leading to tubular atrophy and tubulointerstitial fibrosis (Ziyadeh F et al., 1993 and Hansen K et al., 1976). Hyperglycemia, the significant characteristic of T2DM, induces up-regulation of certain growth factors and their receptors in renal cells. This in turn stimulates both growth and the production of extracellular matrix proteins (Sharma K et al., 1997 and Wolf G et al., 1995) ultimately, contributing to ischemic and fibrotic changes. Links between renal growth and the consequent development of irreparable loss of renal function have long been suspected in the course of diabetic nephropathy (Osterby R et al., 1975, Osterby R et al., 1972 and Mauer J et al., 1984). Because the tubulo-interstitium is the major part of kidney (Nath K et al., 1992 & Schwieger J et al., 1990), tubular hypertrophy is quantitatively
Introduction

Long standing hyperglycemia worsens the situation and it is presumed that the major structural and functional alterations occur in absolute clinical silence. Thus, the early reversible stage ultimately leads to end stage renal disease.

responsible for the largest component of renal growth (Schwieger J et al., 1990 and Wolf G et al., 1995). Renal cells have the capacity of regeneration and thus as a normal mechanism it will tend to resist the tubular changes making the phase reversible.

In the structural alteration phase if unnoticed and untreated it subsequently reaches glomerular damage phase. At nephron level in the glomerulus, glomerular hyperfiltration and hyperperfusion occur before the onset of any detectable clinical changes. Following this thickening of the glomerular basement membrane, glomerular hypertrophy, and mesangial expansion take place (Dronavalli S et al., 2008). During this early hypertrophic stage, glomeruli show regular structure. Significant structural changes particularly thickening of glomerular basement membrane and mesangial expansion occurs after several years of diabetes duration (Lehmann R et al., 2000). The glomerular hyperfiltration and hyperperfusion is due to decreased resistance in the afferent and efferent arterioles of the glomerulus. Many other factors like prostanoids, nitric oxide, vascular endothelial growth factor TGF-β1, and the renin–angiotensin system, specifically angiotensin II showed their involvement in glomerular basement membrane damage (Dronavalli S et al., 2008). Following hyperfiltration the mesangial expansion contributes to the complete renal decline. Hyperglycemia is a fundamental aspect in the development of diabetic nephropathy. The mesangial cell maintains glomerular capillary structure for modulation of glomerular

Figure 1.4 Structural and functional changes in various stages of diabetic nephropathy
filtration. Chronic diabetes is associated with an increase in mesangial cell proliferation and hypertrophy, as well as increased matrix production and basement membrane thickening (Harris R et al., 1991 and Heilig C et al., 1995) and mesangial cell apoptosis (Mishra R et al., 2005 and Lin C et al., 2006). An over-expression of glucose transporters, such as GLUT1 and GLUT4, increases glucose entry into the cells which leads to the expansion of mesangial cells (Heilig C et al., 1995). Three vital mechanisms non-enzymatic glycosylation that generates advanced glycosylation end products, activation of PKC, and acceleration of the aldose reductase pathway explains tissue damage due to hyperglycemia (Friedman E et al., 1999 and Porte D et al., 1996). Oxidative stress amongst them is the common to all three pathways (Brownlee M et al., 2001). The decrease in heparan sulfate proteoglycan (protein providing the negative charge to GBM) is directly proportional to the increase of glomerular matrix proteins (Nerlich A et al., 1991). Hence, such negatively charged proteoglycan provides an anionic barrier to the glomerular basement membrane, and thus its loss offer glomerular permeability to albumin. Thus, albumin leakage is possible only due to glomerular damage.

Proteinuria is one of the major components found due to renal alterations. Proteinuria determination is a routine analysis carried out in renal disease patients. A variety of techniques is used for characterization of urinary proteins and peptides for the early detection of the condition. Further, this would help in improved prognosis and closer monitoring of response to therapy. Comparison of protein patterns between healthy individuals and patients with the disease is increasingly being used to discover biomarkers that could further elaborate disease pathogenesis. The current urine analysis of protein measures either the total level of urine protein or a single protein species. Emerging proteomic technologies allow examination of the patterns of multiple urinary proteins.

There are studies reporting excretion of an abnormal amount of proteins other than albumin in urine during the early course of diabetic nephropathy. Normally due to charge selectivity and pore size of the glomerular basement membrane, proteins of molecular weight < 40 KDa has a free passage through tubular reabsorption and glomerular basement filtration (Barratt J et al., 2007). The average proteinuria level is < 30 mg/24 hrs. But there are studies reporting the presence of such low molecular weight proteins in the early course of diabetes for prediction of diabetic nephropathy correlating significantly with the degree of proteinuria. Now a day’s low-level albuminuria is one of the characteristic of early renal disease exemplified between 30 and 300 mg/day known as microalbuminuria. Protein excretion...
higher than 300 mg/day represents overt proteinuria or macroalbuminuria (Viberti G et al., 1982). Pathological proteinuria is of 3 types 1.) glomerular proteinuria, 2.) tubular proteinuria and 3.) overload proteinuria (Abuelo J et al., 1983). Glomerular alterations due to the permeability of the glomerular capillary walls result in proteinuria especially albuminuria and other high molecular weight proteins (Hong C et al., 1998). Tubular proteinuria is due to reduced reabsorption of proteins present in the glomerular filtrate or due to excretion of proteins derived from injured tubular epithelial cells. It is usually caused by tubulointerstitium alterations. Overload proteinuria is due to the inability of proximal tubules to reabsorb excess of low-molecular-weight proteins. ‘Tubular proteinuria often produces less total urinary protein than glomerular the disease (<2 g/day) due to lower molecular masses of the proteins affected. In healthy individuals, tubular proteins make up 19% of total urinary protein (Barratt J et al., 2007 and Zhou H et al., 2006).

Proteinuria is a marker of well established glomerular injury. Hence, proteinuria predicts the chronic nephropathies efficiently. Proteinuria includes both low molecular weight proteins and high molecular weight proteins. The protein overload provides a toxic environment to proximal tubules. Debate on albumin overload eliciting several responses to tubular cells but in-vitro studies ignore it. Rather compounds that are bound to albumin, such as free fatty acids have been implicated as causative agents in proximal tubular cells injury (Schreiner G et al., 1995). The proximal tubule has receptors for the normal uptake of various ultrafiltered proteins. These receptors are found on brush border region of tubules and uptakes both low as well as high molecular weight proteins (Haymann J et al., 2000 and Braun M et al., 2004). Megalin and cubulin are one of the major glycoprotein receptors (Birn H et al., 2006). Hence, the primary reabsorption at proximal tubules is carried out by the receptors present at the brush border of proximal tubules.

Urine plays a vital role in clinical diagnostics. It is produced by the kidney allowing elimination of waste products from the blood. The human kidney is composed of millions of functional unit called nephrons, which can be divided into two functional parts the glomerulus and the renal tubule. The glomerulus filters whole plasma producing primitive urine, which is further reabsorbed by the renal tubules, and final urine is stored in the bladder. All the metabolic waste is carried away by blood to the kidney for filtration and, therefore, urine may contain information not only from the kidney and the urinary tract but also from more distant organs. Urinary proteome directly reflects any alterations in the kidney or the urogenital tract. In healthy individuals, 70% of the urinary proteome originates from the
kidney and the urinary tract, whereas the remaining 30% represents proteins filtered by the glomerulus (Thongboonkerd V et al., 2005). Thus, analysis of the urinary proteome allows the identification of biomarkers for both urogenital and systemic diseases.

Urine has gained attraction of many researchers because it is available non-invasively in large quantities and depicts both systemic as well as renal tract abnormalities. Urine as a biofluid has gained a reputed position in the studies of clinical proteomics. Urine is very easy to collect and store. It can be stored at -20°C for years without a change in its proteomic composition. The field of urinary proteomics is now shifting from the discovery phase into an era of urinary biomarkers validation in large cohorts. Till today, highly abundant urinary proteins and peptides are extensively studied, but analysis of the less abundant and naturally existing urinary proteins and peptides remains a challenge. Techniques have now been developed for accurately studying such low abundant proteins (Decramer S et al., 2008). Urine has both pros and cons of using it as a source of biomarker discovery. Some of the advantages and disadvantages are mentioned below (Decramer S et al., 2008)

Advantages in comparison to other body fluids and urine’s several characteristics make it a preferable choice for biomarker discovery:

1) It can be obtained in large quantities with non-invasive procedures allowing it’s re-sampling for the same individual. Hence, it helps in the standardization of protocol for obtaining reproducibility and any further improvement in the protocol for obtaining the optimum protein profiling.

2) Urinary peptides of molecular mass <30 KD are soluble in urine and hence, can be analyzed in a mass spectrometer without additional manipulation due to tryptic digests.

3) The urinary proteins/peptides are relatively stable probably due to the storage of urine in the bladder for a longer duration; hence by the time of urine voiding proteolytic degradation due to endogenous proteases is mostly complete. Furthermore, urinary proteome showed no change even when it was stored up to 3 days at 4 °C or up to 6 hours at room temperature (Schaub S et al., 2004 and Theodorescu D et al., 2006).

Disadvantages—Urine has some major disadvantages
1) The protein and peptide concentrations are affected by the fluid intake. However, this is avoided by taking morning sample for obtaining the most concentrated form of proteins/peptides.

2) Hence, variation in disease-specific biomarkers can be observed due to the diurnal variation in the proteome concentration.

Despite of all this urine is considered to be a potent source of biomarker because it directly reflects renal, urogenital tract and systemic changes directly. Hence, urine has emerged mainly as one of the noteworthy body fluids in the field of clinical proteomics as well as biomarker discovery with brisk acceptance and application in the clinics (Decramer S et al., 2008).

Clinical diagnostics and biomarker discovery for early prediction of diseases are the chief focus of current clinical proteomics (Colantonio D et al., 2005 and Hanash S et al., 2004). Today, gel-based techniques are the most frequently and efficiently used methodologies in studying urinary proteomics. All known mass spectrometry techniques are used for urinary proteome analysis which includes, two- dimensional gel electrophoresis-Mass Spectrometry (2DE–MS), Liquid Chromatography- Mass Spectrometry(LC-MS), Surface-enhanced laser desorption/ionization time-of-flight- Mass Spectrometry (SELDI-TOF-MS), Matrix-assisted laser desorption ionization time-of-flight- Mass Spectrometry (MALDI-TOF-MS) and Capillary electrophoresis- Mass Spectrometry (CE-MS) (Thongboonkerd V et al., 2007). The ideal sequence for biomarker discovery would be mass spectrometry-based discovery followed by Enzyme Linked Immuno Sorbent Assay/Western Blotting based validation and clinical application.

Recent development targets the low abundant low molecular weight urinary proteins/peptides. A novel and proficient approach are applied for studying such veiled proteome. For concentrating quantity of such low abundant protein, Proteo-Miner technique is used. It enriches such low abundant proteins (Decramer S et al., 2008) and the procedure is composed of a combinational library of hexameric peptide ligands that are bound to porous polyacrylate beads named “Proteo-Miner”. Each bead is captured with numerous copies of an exceptional hexapeptide ligand distributed throughout its porous structure. Ligands of each bead are potentially different from every other bead. The ligand is generally of known species of proteins. Once all such abundant protein species, present in the urine saturates their binding sites, the remaining unknown molecules can be eluted out and can be enriched and
explored via different efficient techniques (Decramer S et al., 2008). Other than this, various techniques like centrifugation, ultracentrifugation, protein depletion by immuno-affinity, urinary chromatography, and gel fractionation methods are used for concentrating such low abundant proteins. Centrifugation and ultracentrifugation techniques use different solvents and combination of solvents to enrich such minor proteins (Thongboonkerd V et al., 2007 and Thongboonkerd V et al., 2006). Immunoaffinity is used to deplete the high abundant proteins like albumin, IgG, anti-trypsin, IgA, haptoglobin and transferrin by binding with their specific ligands in the columns and hence can be removed. However, the smaller proteins can be eluted and concentrated for further procedure (Rao P et al., 2007). But this technique has a drawback of removing some low molecular weight proteins, which tend to bind with such abundant proteins as a normal physiological mechanism. Urinary chromatography and gel filtration methods follow the same technique of immuno-affinity and cation exchange method. Once such rare proteins are enriched, they are then separated, isolated, sequenced (recognized) and studied further for pathophysiology and biochemistry of the disease.

One of the majorly used clinical approaches for the isolation of desired proteins/peptides is Two-dimensional gel electrophoresis (2DE) or Two-dimensional in-difference gel electrophoresis (2DIGE). These techniques are easy to perform and are carried out in most of the proteomic laboratories. However, it has some limitations (i) the technique as whole and spot analyses are laborious (ii) it can be impractical for a large number of urine samples due to inter and intra individual variability (iii) to achieve good result low abundant proteins should be concentrated and salts must be removed before analysis (Thongboonkerd V et al., 2004); (iv) a substantial volume of urine from every individual is required, thus, space for storage at -20 °C to -80 °C is a problem (v) recovery of hydrophobic proteins is challenging (Rabilloud T et al., 1999) and (vi) visualization of isolated proteins depends on sensitivity of the stain and amount of protein resolved (Lopez M et al., 2000 and Klein E et al., 2004). Regardless of all above mentioned limitations, protein/peptide species can be isolated after optimization and standardization of the protocol.

This type of analysis has a very less sample requirement; it gives high throughput and due to its automation they are acquired by many proteomics laboratories as a routine proteomic procedure. Hence, different methods coupled with high-end mass spectrometry, array formats, capillary electrophoresis or to chromatography are developed. The surface-enhanced laser desorption/ionization (SELDI) technique is one of the examples of such high throughput
advanced urine analysis techniques. Without former concentrating or precipitating urinary proteins, direct native urine can be used on SELDI Protein Chip (Schaub S et al., 2004 and Hampel D et al., 2001). The bound proteins may then be directly analyzed by MALDI-TOF-MS. Also, capillary electrophoresis (CE) coupled with the powerful detection capability of the mass spectrometry along with time-of-flight provides a high-resolution sequence of urinary proteins. Liquid chromatography coupled to mass spectrometry (LC-MS) also proposes a sensitive alternative for low abundant urinary proteome analysis. Thus, the above-mentioned gel free techniques endow with the characterization of single proteins or protein profiles of disease-specific biomarkers which can be validated through clinical based studies. The advantages and disadvantages are mentioned in table 1.1.

Table 1.1 Summary of advantages and disadvantages of different MS-based proteomic technologies

<table>
<thead>
<tr>
<th>Technology</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>2DE-MS</td>
<td>Widely available, applicable to large molecules</td>
<td>Time-consuming, technically demanding, difficult analysis for smaller protein (&lt;10 kDa) and highly hydrophobic proteins</td>
</tr>
<tr>
<td>SELDI-MS</td>
<td>Easy process, completely automated, capable of high throughput, low sample volume required</td>
<td>Low reproducibility, lack of comparability, low-resolution MS</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Highly sensitive, completely automated, multidimensionality</td>
<td>Time-consuming, sensitive to interfering compounds</td>
</tr>
<tr>
<td>CE-MS</td>
<td>High resolution, high selectivity, fast separation, automation, low cost</td>
<td>Not well suited for the analysis of high-molecular-weight proteins (&gt;20 kDa)</td>
</tr>
</tbody>
</table>

Various mass spectra based techniques are used for detecting the low abundant proteins/peptides. These peptides/proteins can be used for the early detection of any disease or condition.

A urine derived biomarker is proficient because of being simple, noninvasive and can directly reflect the site of injury. Decades ago it was regarded that glomerular damage was the earliest manifestation of diabetic nephropathy, but now studies have explored the possibility of tubular damage as an early sign of diabetic nephropathy. It gradually involves glomerular damage and the reversible renal damage is then transformed to irreversible renal damage. Excessive excretion of protein in urine is called proteinuria, which results due to different abnormalities like increased permeability of the glomerular basement membrane to high molecular weight such as albumin; decreased / increased secretion of low molecular weight
proteins filtered by the proximal tubular cells and finally increased concentration of low-molecular weight (LMW) proteins that are freely filtered by the kidney (Barratt J et al., 2007). Thus, in our study we concentrated on proteins which are excreted during tubular overload phase which are competent enough to predict early stage diabetic nephropathy.

Tubular damage urinary biomarkers comprises of urinary enzymes or low molecular weight plasma proteins. Their increased excretion in urine is due to their impaired reabsorption at tubular cells, or due to their direct secretion/release in urine tubular epithelia cells (Barratt J et al., 2007, Hong C et al., 1998 and Flynn F et al., 1990). We have discussed in detail regarding some of the tubular biomarkers like, retinol-binding protein (RBP), α1-microglobulin (A1MG) and β2-microglobulin (B2MG), are used extensively for diagnosing and monitoring tubular damage in various renal diseases (Barratt J et al, 2007 and Hong C et al., 1998). Furthermore, some urinary enzymes are also utilized to study renal tubular damage, amongst which studies on N-acetyl β-D-glucosaminidase (NAG), γ-Glutamyl Transpeptidase (GGT) and alanine aminopeptidase (AAP) are going on (Hong C et al., 1998). All the previous mentioned enzymes are high-molecular weight and cannot cross the glomerulus barrier but they originate from the renal tubular cells and excrete directly in urine (Hong C et al., 1998 and Matheson A et al., 2010). Hence, they can be used for prediction of renal tubular damage due to type 2 diabetes mellitus.

NAG is the hydrolytic Lysosomal enzyme and having a very low physiological activity and has a molecular weight of 150 K Da (Skalova S et al., 2004). The particular enzyme principally originates in proximal tubule and does not pass through the filtration barrier but due to hyperglycemic condition the epithelial cells of tubules will be stressed and the Lysosomal activity of the cells will be increased and will start excreting in urine and thus the early onset of the condition can easily be evaluated. NAG has been reported to be a very sensitive and reliable marker for renal failure. NAG is exclusively distributed most widely in nephron and released and excreted in urine due to tubular damage (Pai P et al., 1998). Urinary excretion of renal tubular enzymes and low molecular weight proteins have been recommended as useful markers for detection of minor changes in proximal tubular function long before elevation in other markers as proteinuria and rise in serum creatinine. Thus NAG excretion provides a useful non invasive and easy to repeat test in assessing the initial malfunction or damage of the proximal tubular epithelial cells in early stages of progressive disease (Bazzi C et al., 2002). There are studies carried out which shows a clear correlation between NAG, poor metabolic control in diabetics and signs of nephropathy (Agardh C et
Increased NAG amount in urine can predict the development of microalbuminuria in subjects with type 1 diabetes along with proximal tubular injury (Kordonouri O et al., 1998). Hence, urinary NAG can prove to be a very effective prognostic and diagnostic marker for diabetic nephropathy. Another marker which can be proved to be very important is Gamma Glutamyl Transferase (GGT) and is located exclusively among the proximal tubular brush border (Miller S et al., 1976) membrane of the nephrin thus when the tubular cells are damaged, they release GGT into ultrafiltrate and thus urinary enzyme activity increases. Alanine Aminopeptidase (AAP) is also a brush border enzyme and is involved in amino acid metabolism. Excretion of AAP reflects renal dysfunction and tubular damage (Price R et al., 1982). Additionally excretion of urinary enzymes varies with the activity of the renal disease and therefore they can be used to monitor the rate of recovery. NAG, AAP and GGT are reported to be sensitive indicators of renal damage and can be used as diagnostic biomarkers for early progression of diabetic nephropathy.

Kidney injury molecule-1 is a newly emerged biomarker and is described as renal tubular cell dedifferentiation and injury marker (Mishra J et al., 2005). Urinary KIM-1 is type 1 cell membrane glycoprotein. A study carried out suggests that KIM-1 can be used to diagnose acute renal failure i.e. in diagnosis of early renal manifestation (Liangos O et al., 2007). β2 microglobulin, a globular polypeptide has a molecular weight of 11.8 K Da. It is not stable in acidic urine and starts degrading at pH 5.5. It is a low molecular weight marker and normally it is being excreted in urine but is reabsorbed back. In type 1 diabetes patients urinary excretion of β2 microglobulin was higher compared to other groups (Togores S et al., 1991, Asami T et al., 1992). It is a well known fact that serum β2 microglobulin level is usually >30 fold higher than normal healthy adults (Gejyo Fet al., 1986). Thus, it is a good predictor of tubular damage in diabetic nephropathy in insulin dependent diabetic patients. Retinol binding protein is a low molecular weight protein of 21.4 K Da and is generally bound to transthyretin of molecular weight 55 K Da but the unbound fraction can easily cross the glomeruli filter and completely reabsorbed back and then completely catabolized in normal condition (Parviainen M et al., 1983). It is stable at acidic pH and different temperatures (Blumsohn A et al., 1990). It is a marker of proximal tubule damage (Bernard A et al., 1981) and high concentration of urinary excretion is observed in type 1 diabetic patients. It has a significance of excreting before in urine than the appearance of microalbumin. Pfleidrer et al., reported that there is a significantly excretion of alpha1 microglobulin in the early stages of the disease process and microalbumin was still not detected (Pfleiderer S et al., 1993). It is a
27 K Da glycoprotein present in various body fluids and as not being an acute phase protein it is stable in a broad range of physiological condition and can be used for early detection of tubular dysfunctioning. Tan et al showed the efficacy of a low molecular weight marker of 13 K Da, Cystatin C for accurate, estimation of glomerular filtration rate in type 1 diabetes (Tan G et al., 2002). Cystatin C is a good marker of GFR (Andersen K et al., 1994). It is non glycosylated protein belonging to family of cysteine protease inhibitors (Grubb A et al., 1982). It is produced by all nucleated cells and is freely filtered through glomeruli but is reabsorbed and catabolised again (Lofberg H et al., 1979) and it is shown that cystatin c is a new promising biomarker for determination of early impairment of renal function in insulin dependent diabetes (Newman D et al., 2002).